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Page 87, paragraph 5, last line, *read* plating *for* planting.

Page 307, paragraph 4, line 1, *read* mode *for* node.

Page 435, *transfer* line immediately following footnote 1 to *follow* line 4 of paragraph 2.

Page 518, *transfer* line 7 of paragraph 3 to *follow* line 3 of paragraph 4.

July number, Table of Contents, line 20, *read* Stark *for* Starr.

Page 655, *transfer* line 11 to line 10 of paragraph 2.

Page 742, paragraph 2, line 13, *read* transmission *for* subsequently.

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[1] Any errata appearing in the December, 1942, number will be published in Vol. 33, No. 2.

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[*] A package of cards bearing the following index entries for volume 32 was inadvertently mislaid and only recently recovered. These entries are therefore here published as a supplemental index for last year's volume, with regrets for the delay and for the inconvenience to others thus occasioned.—Frederick V. Rand.

[†] This portion of the index may be removed and bound with the 1942 index.

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ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT THE THIRTY-THIRD ANNUAL MEETING OF THE SOCIETY, DALLAS, TEXAS, DECEMBER 29, 1941, TO JANUARY 1, 1942

The Relation of Potassium to Water-soaking of Tobacco. ALLINGTON, W. B., AND JAMES JOHNSON. The predisposing effect of a low potash nutrition of plants on the severity of certain diseases has long been known or suspected. Studies on physiological water-soaking have shown that this condition also is an important predetermining factor for natural infection with the organisms of tobacco blackfire (*Bacterium angulatum*) and wildfire (*B. tabacum*). Continued investigation of physiological water-soaking has shown that tobacco plants of field size, which have developed or are approaching potassium-deficiency symptoms, water-soak readily; while plants of a similar size, with ample potassium, water-soak only with great difficulty under conditions of high relative humidity and low light intensity. Certain other factors such as nitrogen supply may play an important part in water-soaking as well. Potassium-starved plants, therefore, are predisposed to water-soaking and, subsequently, to bacterial infection. A shortage of potassium in leaf tissue is known to induce necrosis of isolated cells and finally large groups of cells. Since living tissue surrounding necrotic areas in leaves has repeatedly been observed to water-soak more readily than other tissues, this behavior may explain water-soaking when influenced by low potassium. (Wisconsin Agricultural Experiment Station and Division of Tobacco Investigations, Bureau of Plant Industry, U. S. Dept. of Agriculture, cooperating.)

Two Leaf-spot Diseases on Sorghum and Related Grasses. BAIN, D. C., AND C. W. EDGERTON. A leaf-spot disease caused by an apparently undescribed fungus has been found on sorghum, Johnson grass, Sudan grass, and a variety of sugar cane (C.P. 33–243) in Louisiana; on sorghum and Johnson grass in Mississippi; and on Sudan grass at Arlington Farm, Virginia. The disease shows in the form of large zonate spots. Spores in pinkish masses are produced in great abundance. The fungus has been isolated from leaves and seeds and the disease has been produced by inoculation. The fungus does not fit well into any of the described genera. Tentatively, as suggested by C. L. Shear, it is being placed in the new genus *Gleocercospora*. This fungus has been confused with *Titaeospora andropogonis* in this country (Pl. Dis. Rptr. 25: 142. 1941), and possibly in plant disease reports from other parts of the world. *T. andropogonis* has been observed on Johnson grass in Louisiana and Mississippi.

nom.

New Species of Sphaceloma on Myrtaceae. BITANCOURT, A. A. Since the discovery of *Elsinoë pitangae* on Surinam cherry (*Eugenia pitanga*) the imperfect stage (*Sphaceloma*) of the genus has been found on other fruit trees of this family of phanerogams, *i.e.*, *jaboticaba* (*Myrciaria jaboticaba*) and guava (*Psidium guajava*) both collected by the writer in the State of São Paulo, Brazil, and on plants native to southern Chile, murta (*Ugni lanceolata*) and arrayán (*Myrceugenia apiculata*), collected by S. Arentsen. On the five hosts the pathogens cause the anthracnose type of lesion. The other type of disease caused by the Elsinoaceae is referred to under the more or less common term ''scab.'' Scab of *Eucalyptus* spp., however, a hitherto unreported disease of this important member of the Myrtaceae, appears not to be caused by this group of pathogens. A species of *Coniothecium* was isolated from the diseased tissues.

Antagonism between Strains of the Peach-mosaic Virus in Western Colorado. BODINE, E. W. Preliminary studies of the strains of the peach-mosaic virus in western Colorado show definite indications of ''antagonism'' between strains. An Elberta peach tree affected with the slight strain of the virus when re-inoculated with buds of the severe strain in the fall of 1938 showed only symptoms of the slight strain the following spring. After buds from the re-inoculated tree had been inserted into 20 healthy Elberta test trees in the fall of 1939, 10 of these trees showed symptoms of the slight strain the spring following inoculation. The remaining 10 trees showed very slight or no symptoms. The inserted buds made growth unions in 18 cases. Ten of these inoculated trees, 5 trees expressing symptoms of the slight strain and 5 showing very slight to no symptoms were then re-inoculated with the severe strain of the virus in the spring and the fall of 1940. In the spring of 1941, all 10 cases expressed only the symptom apparent in each tree in 1940. The remaining 8 inoculated trees used as controls showed the same symptoms as those manifested in 1940. For each year's study adequate control inoculations were made in order to insure that the virus of severe mosaic was introduced in each instance. Alternate buds were taken from the same bud stick for both the control and re-inoculation tests.

Environment and Plant Disease in the Far Southwest. BROWN, J. G. The far Southwest is a region of contrasts. Elevations vary from approximately sea-level to two or more miles above; extremes of temperature range from – 30° F. to 120° F. or even higher; annual precipitation fluctuates between 3 and 20 inches; soils, predominantly alkaline, may be neutral or somewhat acid at the higher elevations and, physically, range from porous to impervious and from almost pure pulverized granitic rock to clays and loams satisfactorily supplied with organic matter; air movement, predominantly toward the west in large masses in summer and toward the east in winter, becomes locally very complex because of the rough topography and the alternation of vegetative cover with bare soil; evaporation exceeds precipitation in the ratio of 5: 1 to 29: 1. These environmental factors may exert a marked influence on the kinds, prevalence and destructiveness of plant disease. This paper presents a few of the many striking examples that might be given to illustrate relations between environmental factors and plant disease in this Southwest country of contrasts.

Gross Pathogenic Effects of Pythium graminicolum, Pythium debaryanum, and Helminthosporium sativum on Seedlings of Crested Wheatgrass. BUCHHOLTZ, W. F. When placed in steamed soil above the seed, immediately below the seed, 1 inch, and 2 inches below the seed of crested wheatgrass (*Agropyron cristatum*), *Pythium debaryanum* interrupted germination, except at 2 inches below the seed, apparently had little or no effect on seedlings. *Helminthosporium sativum* interrupted germination when near the seed; occasionally, at 1-inch and 2-inch depths, was variable in its effect on seedlings, but tended to be somewhat like *P. debaryanum*. *Pythium graminicolum* in all cases interrupted germination, pruned roots, and induced stunting and death of seedlings. When placed in steamed soil alongside rows of seedlings up to 1 month of age, *P. graminicolum* killed seedlings up to 3 weeks of age, induced wilting and stunting of seedlings of all ages. *P. debaryanum* and *H. sativum* killed only occasional seedlings 1 week old or less, induced no detectable wilting or stunting. In brome-grass sod, 1 inch below the seed, *P. graminicolum* interrupted germination, pruned roots, and induced wilting, stunting, and death of seedlings. *P. debaryanum* and *H. sativum* induced no detectable symptoms. The symptoms induced by *P. graminicolum* in these experiments were typical of seedling blight of crested wheatgrass in field soil. *P. graminicolum* is considered to be the cause of the disease. Isolates of *Fusarium* spp. in preliminary trials were less pathogenic than any of the 3 fungi used in these comparisons.

Necrotic Spot, a Peach Disease Transmissible by Budding. CATION, DONALD. A Windsor cherry tree showing no symptoms, budded to Elberta and Carmen peach trees in 1939, resulted in foliar necrotic spots in 1940. Inoculations in 1940 from cherry to peach and from peach to peach resulted in similar symptoms in Hale, Golden Jubilee, South Haven, and Halehaven. Faint chlorotic spots, accompanied by mild leaf distortion, slightly retarded foliation, and a trace of spot necrosis were evident on several varieties in the early season of 1941. Affected leaves were most evident in mid-July. At that time light-brown, dead membranous areas appeared in unfolding leaves. The affected areas soon fell out, leaving clean-edged holes, and the leaves remained green and persisted throughout the remainder of the season. Die back, cortical necrosis, and ring spotting were not evident, differentiating the disease from the ring spot of peach reported by Cochran and Hutchins (Phytopathology 31: 860, 1940). Trees infected in 1940 showed some, but proportionately fewer, affected leaves in 1941.

Variability in Response of Flax to Seed Treatment. CHRISTENSEN, J. J., AND M. B. MOORE. From 1936 to 1941 about 700 lots of flax seed obtained from farmers in Minnesota were tested for response to fungicides. Yield tests were made at University Farm and at 4 branch stations. In most cases, seed treatment increased the stand and vigor of seedlings and often increased yield, although in some cases it resulted in a decrease. The value of seed treatment varied greatly with the season and with seed lots of the same variety. Seed of the same variety produced in 5 regions in Minnesota during the same season responded quite differently to seed treatment. Different seed lots of the same variety, whether treated or not, possessed different yielding abilities. The results indicate that the value of seed treatment depends on variety, source, and condition of seed, type of soil flora, sometimes flora of seed, environmental factors at time of seeding, and on the fungicide used. Ceresan and New Improved Ceresan were the most effective fungicides tested. These results help to explain the conflicting reports on the effects of seed treatments of flax.

Isolation of Bacterium angulatum from Overwintered Tobacco Field Soil. DIACHUN, STEPHEN, W. D. VALLEAU, AND E. M. JOHNSON. To determine whether *Bacterium angulatum* is present in overwintered soil in tobacco fields, samples of the soil were mixed with sterile water and poured onto the lower surface of susceptible tobacco leaves which were artificially water-soaked just before inoculation. Water-soaking of the leaves permits bacteria present in water on the leaf surface to enter the leaf through stomata, and produce infection. Continued water-soaking is not necessary. Leaves were water-soaked by forcing a strong stream of water from a hypodermic syringe against the lower surface, the end of the needle of the syringe being held approximately one inch from the leaf. Between November 2, 1940, and April 18, 1941, *Bacterium angulatum* was recovered from field soil in which naturally infected tobacco had grown during the summer of 1940. One hundred eighty-two samples collected on 15 different dates were tested; *Bacterium angulatum* was isolated from 37 of these samples in 9 of the 15 tests.

The Reversal Effect: Why Fungicides Change in Rank in Repeated Field Tests. DIMOND, ALBERT E. The ranking of any fungicide may change radically in repeated tests. Many such changes may result from the reversal effect. If a series of dosages of each material is applied, a straight line relating fungicide dosage to disease control is obtained on logarithmic-probability coordinates. Under comparable conditions, the slope and LD95 of these curves are properties of the fungicide. When slopes for the dosage-control curves of 2 fungicides differ, these curves must cross one another. At the intersection point, the 2 materials have identical disease-controlling properties. Above this point, the material having the steepest slope is most effective; whereas, below this point, the material having the flattest slope is most effective by any method of comparison. Environmental changes influencing disease development will alter the slope of the dosage-control curve. From test to test, therefore, the point of intersection of dosage-control curves of the same 2 fungicides may be expected to change. If, in one test, the investigator compares his materials at a single dosage above the intersection point of their dosage-control curves, and, in a second test, compares them below this point, inversion in order of effectiveness of the materials can be explained.

Vacuolar Inclusions in Cells of Sugar Cane Affected with Chlorotic Streak. DUFRENOY, JEAN. Split stalks of sugar cane affected with chlorotic streak show red discolorations in the tracheary vessels, mostly at the nodes. In the parenchyma cells surrounding the vessels, spherical bodies having the structure of ''Coacervates'' are frequently observed. Surrounding each body is a membrane, apparently of phosphatides, as it yields molybdenum blue with molybdenum reagents and stains blue with nascent indophenol blue. The membrane encloses a dense colloidal mass rich in catechol and catechol oxidase, as demonstrated by the rapid oxidation of para- or ortho-phenols and the rapid absorption of neutral red, safranin, and other basic dyes used as vital stains. Coacervates, however, have also been observed in cane free of chlorotic streak and commonly in lesions of red rot (*Colletotrichum falcatum*). They are also common in the tissues of various other plants that are in a diseased or unthrifty condition. They have often in the past been mistaken for organisms of one type or another.

Sterilization of Rhizoctonia Sclerotia with Corrosive Sublimate. ELMER, O. H. A momentary dip of Rhizoctonia-infected seed pototoes in acidulated 3–500 concentration $HgCl_2$ sterilizes adhering sclerotia more effectively than does a 10-minute treatment in the concentration 1–500. Fifteen comparative tests since 1936 resulted in 74.7 per cent Rhizoctonia-free sprouts following the momentary dip and 64.2 per cent Rhizoctonia-free sprouts following the 10-minute treatment. Nontreated controls averaged 7.3 per cent Rhizoctonia-free sprouts. Soil-borne Rhizoctonia-infection frequencies were not greater than 3.8 per cent. Death of Rhizoctonia sclerotia resulting from the momentary or the 10-minute treatment evidently occurs following planting and not during the treatment period. After sclerotia-covered tubers were treated by the momentary or by the 10-minute treatments, then dried and cut into half, those half-tubers held 14 hours in running water to remove adhering $HgCl_2$ developed sprouts of which 89.1 and 91 per cent, respectively, were infected with Rhizoctonia. The unwashed half-tubers developed sprouts of which only 28 and 41.5 per cent, respectively, became infected. Effective Rhizoctonia control evidently requires a sufficient dosage of $HgCl_2$ on the planted tubers, either applied momentarily in high concentrations or during a soak treatment at lower concentrations. Momentary dipping in acidulated 1–500 $HgCl_2$ was ineffective.

Dormant Applications of Lime Sulphur for Controlling Raspberry Anthracnose. ELMER, O. H. Spring applications of commercial liquid lime sulphur to anthracnose-infected dormant black raspberry canes (6-year experiments) resulted in the following percentages of infection on subsequently produced new canes: anthracnose-free, 35.8; canes with occasional isolated lesions, 46.3; medium infection, 13.8; severe infection, 4.2. Infection percentages occurring on the new canes from nonsprayed controls were: anthracnose-free, 4.8; occasional isolated lesions, 10.3; medium infection, 31.7; severe infection, 57.6. Consecutive annual applications of only a dormant spray practically eliminated anthracnose from an experimental raspberry field. A November application of

1–10 lime sulphur to the canes was ineffective for controlling anthracnose. An application made just prior to the appearance of leaves was more effective than one made a month earlier. Lime sulphur is arrarently more lethal to the anthracnose organism in the old cane lesions when the perithecia approach maturity than earlier in the dormant season, when they are still immature. Control is evidently due to prevention of sporulation through sterilization of old cane lesions and not to preventive applications on the new canes. Both Bordeaux 3–6–50, applied as a dormant spray, and lime sulphur 1–40, applied after leaves have appeared, were ineffective for preventing anthracnose.

Attenuation of Phymatotrichum omnivorum Cultures by Repeated Transfer of Young Mycelium. EZEKIEL, WALTER N. Successive transfer of cultures of *Phymatotrichum omnivorum* during the relatively early development of the colony causes rapid attenuation. There is extreme reduction in rate of growth, and, eventually, nearly complete failure of growth, making further transfer impossible. Attenuation has resulted from repeated weekly transfer from agar plates of peripheral discs (at 4 cm. from the original inoculum, and thus carrying mycelium only 1–2 days old) as compared to transfer of proximal discs (within 1 cm. of the inoculum, and thus carrying mycelium 4–5 days old). Proximal transfer lines retained somewhat vigorous growth, although inferior to that of lines transferred similarly but at 3-week intervals, while peripheral lines declined rapidly. Preliminary experiments indicate that the immediate cause of the attenuation is probably neither accumulation in attenuated cultures of diffusible inhibitory materials (as a phage) nor lack of the growth-promoting materials that have as yet been tested.

Cotton Root Rot, the Weather, and Cotton Yields. EZEKIEL, WALTER N. Within certain limits, increased rainfall during the several months preceding harvest leads to corresponding increase in the prevalence of root rot, caused by *Phymatotrichum omnivorum,* but additional rainfall during this period also favors growth of the cotton plant. Losses correctly attributable to root rot may be masked in average figures by increased yields from surviving plants and particularly the much higher yields in fields where the disease is not prevalent. For entire counties, average yields per acre, therefore, generally increase with greater prevalence of root rot, to a peak with around 25 per cent root rot. Above this, county-yield figures decline. For example, in Bell County in 1939, 10 per cent root-rot prevalence accompanied an average yield per acre of 135 lb.; 14 per cent in 1938, yield 157 lb.; 20 per cent in 1928, yield 184 lb.; and 29 per cent in 1937, yield 146 lb. Disastrous losses from root rot thus may not show from simple comparison of average yields and average prevalence. Several lines of work have instead furnished a basis for estimating percentage reduction in yield as approximating nine-tenths the percentage of plants killed by date of picking.

Tetrachloro-para-benzoquinone, an Effective Organic Seed Protectant. FELIX, E. L. Tetrachloro-para-benzoquinone has been tested further in the greenhouse as a seed protectant against damping-off under conditions of high soil moisture. In 41 tests in which Spergon, a commercial preparation containing 99 per cent tetrachloro-para-benzoquinone as the active ingredient, was applied to peas at a dosage of ¼ per cent of the seed weight, the mean difference in stand was 52.9 per cent and in height of plant 0.85 cm. in favor of the treated, with odds of over 1000 to 1 respectively, 10 days after planting. Active dosage of ⅛ per cent the seed weight in diluted and undiluted form yielded somewhat less stand than the higher dosage, but resulted in slightly taller plants in the diluted form. Addition of derris root, containing 5 per cent rotenone, to Spergon appeared not to reduce the effectiveness of the fungicide, indicating possible compatibility of the two. Spergon treatment of machine-delinted cotton seed at the rate of 3 oz. undiluted or 4 to 6 oz. of 25 per cent Spergon in talc per bushel of seed, effectively controlled damping-off in flats of Mississippi cotton soil.

Infection of Forage Grasses with Flag Smuts of Wheat, Rye, and Grasses (Urocystis tritici, U. occulta, and U. agropyri, respectively). FISCHER, GEORGE W. Inoculations of 32 species of *Agropyron, Elymus, Hordeum,* and *Sitanion* with smut spores of *Urocystis tritici, U. occulta,* and *U. agropyri* resulted in the development of typical flag-smut symptoms on the following grasses: *U. tritici* infected *Agropyron caninum* (2 accessions), *A. repens, A. spicatum* (2 accessions), *A. semicostatum, Elymus glaucus* (2 accessions), and *E. triticoides; Urocystis occulta* infected *Agropyron caninum, A. inerme,* and *Elymus canadensis* (3 accessions); *Urocystis agropyri* (taken from *Hordeum nodosum*) infected *Agropyron caninum* and *Elymus canadensis* (4 accessions). The 3 flag smuts seem to have entirely different host ranges among the grasses used. *Urocystis tritici* and *U. agropyri* are practically identical in their morphology, and it is recommended that they be considered as races of the same species, which, by priority, would bear the name *U. agropyri.* The spores of *U. occulta* seem to be less completely invested by the sterile cells, which character, if it proves to be constant, would distinguish the rye flag smut from that of wheat and grasses. The demonstrated susceptibility of grasses to flag smut of wheat and the morphological identity of this smut with the similar flag smut on grasses suggests a possible explanation of the source of outbreaks of wheat flag smut in the U. S. where flag smut of grasses has long been known from coast to coast on a wide variety of grass species.

Comparative Value of Certain Popular Fungicidal Dusts in Control of Head Smut (Ustilago bullata) and in Improvement of Stands in Forage Grasses. Fischer, George W. Copper carbonate and basic copper sulphate (about 50 per cent copper), formaldehyde dust (Formacide), Semesan (30 per cent Hydroxymerchlorophenol), ''2 per cent Ceresan'' (2 per cent ethyl mercury chloride), and New Improved Ceresan (5 per cent ethyl mercury phosphate) have been given comparative tests at Pullman, Washington, for control of head smut of grasses and for improvement of grass stands. The grasses used were *Agropyron trachycaulum* (slender wheatgrass), *Bromus marginatus* (mountain bromegrass), *B. catharticus* (rescue grass), *Elymus canadensis* (Canada wild rye), *E. glaucus* (blue wild rye), and *Hordeum nodosum* (meadow barley). The copper dusts were generally ineffective in controlling head smut, even at as high a rate as 6 oz. per bushel of seed, and improved stands only slightly. The same results were obtained with formaldehyde dust. Semesan, at 2, 4, or 6 oz. per bushel of seed, gave, generally, greatly improved stands, but very little smut control. In striking contrast to the poor smut control obtained with the above dusts, ''2 per cent Ceresan'' and New Improved Ceresan gave excellent smut control and from 300 to 500 per cent better stands than the untreated check rows. On the basis of these results, either ''2 per cent Ceresan'' at 2 to 4 oz. per bushel of seed or New Improved Ceresan at ½ to 1 oz. per bushel can be recommended where combined head-smut control and excellent stands are desired. Except with *Hordeum nodosum*, the use of these dusts in considerable excess (2–4 times the recommended dosage) did not reduce the stands.

The Inheritance of Pathogenicity in a Cross between Physiologic Races 22 and 24 of Melampsora lini. Flor, H. H. The pathogenicity of 133 F_2 cultures of a cross between physiologic races 22 and 24 of *Melampsora lini* as indicated by the reaction of 17 rust-differentiating flax varieties was determined. J.W.S., immune from both parent races, was immune from the 133 F_2 cultures. Buda, susceptible to both parent races, was susceptible to all F_2 cultures. Pathogenicity to Williston Golden and Williston Brown was inherited as a unit. These varieties, susceptible to both parent races, were resistant to 17 and susceptible to 116 of the F_2 cultures. Ability to produce a virulent infection type on all varieties, except Williston Golden and Williston Brown, was inherited as a recessive character. Pathogenicity to Bombay, Newland, Tammes' Pale Blue, and Ottawa 770 B was independently inherited and conditioned by single pairs of factors. Pathogenicity to Akmolinsk, Abyssinian, and C.I. No. 836 was inherited as a unit in a simple Mendelian ratio, as was pathogenicity to ''Pale-blue crimped'' and Kenya. Pathogenicity to Argentine and Bolley Golden, varieties having 2 pairs of factors for resistance to race 24, and to Italia Roma. C.I. 357–1 was conditioned by 2 pairs of factors. The 133 F_2 cultures yielded 68 physiologic races, 66 of which had not previously been isolated. (Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture and the North Dakota Agricultural Experiment Station.)

Identity of the ''Intermediate'' Mosaic in the Sugar-cane Variety C.P. 28–70. Forbes, I. L. and P. J. Mills. It has been reported previously that in the sugar-cane variety C.P. 28–70 the presence of a green mosaic virus would protect the plant against a yellow mosaic, and *vice versa.* Plants inoculated with mixed viruses would develop either the green or the yellow mosaic symptoms,—but not both. The results were of interest as bearing on immunity. Further inoculation tests with these viruses have revealed that an occasional plant will harbor both viruses and display their respective symptoms. In 5 experiments a total of 295 plants inoculated with juice from stalks showing mixed symptoms gave results as follows: 142 plants without symptoms, 84 with green mosaic, 32 with yellow mosaic, and 37 with mixed symptoms. With the same inoculation technique green- or yellow-mosaic material alone gave rise to the respective symptoms in about 80 per cent of the inoculations. The results suggest an antagonistic action between the two viruses, but, occasionally, they may occur together in the same plant. There is no evidence that the host produces any substance conferring ''immunity.''

Relation of the Hot-water Treatment of Sugar Cane to the Development of Red Rot. Forbes, I. L. Chlorotic Streak of sugar cane may be eliminated from seed cane by immersion in water at 52° C. for 20 minutes. As such a treatment may become advisable as a control measure, studies have been made during the past two years on the effect of such treatment on cane irrespective of this disease. These studies have included the relation of the treatment to the control of the red rot (*Colletotrichum falcatum*), which also is generally distributed throughout the Cane Belt. Tests with varieties Co. 281 and C.P. 28–11, considered resistant to red rot, indicated that the hot-water treatment made these canes more susceptible. Tests with the variety C.P. 33–243, considered susceptible to red rot, indicated that the hot-water treatment made this variety still more susceptible. However, suspensions of spores of the red-rot fungus were not infective after the heat treatment. There was also very little red rot in stalks inoculated 1 to 2 days prior to the hot-water treatment, which indicates that spores disseminated at planting time were destroyed. In cane, given a heat treatment 3 days after inoculation, the fungus was not entirely killed, but its development seemed to have been arrested.

Some Factors Influencing Curly-top-virus Concentration in Sugar Beets. GIDDINGS, N. J. Curly-top-virus concentration is neither easily nor very accurately determined. The most satisfactory method yet found is to feed nonviruliferous leaf hoppers for a short period on the plants or extracts to be tested, transfer them to healthy young sugar-beet plants, and determine the relative percentage of plants that become infected. Living plants were used as virus sources in this work. Feeding periods of 3 to 9 hours were found most satisfactory. The shorter feeding periods gave best results if conducted at a temperature of about 110° F. Highly resistant beets were found to have a significantly lower virus concentration than susceptible ones. The less virulent strains of curly-top virus were present in significantly lower concentrations than the more virulent strains. Beets, infected for several months, showed significantly lower virus concentration than those infected for a few weeks; but it is possible that soil nitrogen may be a factor in this case.

The Fungicidal and Phytocidal Properties of Some Metallic Alkyl-dithiocarbamates. GOLDSWORTHY, M. C., E. L. GREEN, AND M. A. SMITH. Laboratory and field studies to determine the fungicidal and phytocidal properties of sodium, iron, lead, zinc, copper, silver, and mercury dimethyl, diethyl and dibutyl dithiocarbamates indicate that the dimethyl compounds, as a group, the more toxic to spores than those of the diethyl and dibutyl series. The soluble sodium salts were phytocidal, as were some of their metallic derivatives. The copper and mercury salts were unsafe to use on apple, peach, and bean. The lead salts caused no injury to these plants but the iron, zinc, and silver compounds caused some injury under certain conditions. Weathering appeared to change the iron and zinc compounds so that they injured peach leaves. Lead dimethyl dithiocarbamate is the most promising of the group tested. One season's test against apple scab in Maryland and Missouri indicated that iron and lead dimethyl dithiocarbamates could be safely applied to apple varieties and would control scab under the conditions prevailing in 1941. Peach scab and peach brown rot tests in Maryland showed that iron dimethyl dithiocarbamate would control these diseases, but that it may injure foliage and delay fruit maturity. It did not control cherry leaf spot in Maryland.

Compatibility of Diluent Dusting Materials with Copper Fungicides with and without Calcium Arsenate. GOODWIN, M. W., S. L. HOPPERSTEAD, AND K. J. KADOW. Samples of dusts, both with and without calcium arsenate, were prepared, using 48 different diluent materials, each with red cuprous oxide, tribasic copper sulphate and copper oxychloride. These were analyzed for pH, water-soluble Cu, and water-soluble As_2O_5. It is apparent that the pH of the mixture is a good indication of the amount of water-soluble copper liberated. There is a good correlation between pH and water-soluble copper, with the danger point about pH 5.5. Below this pH, amounts of water-soluble copper are liberated that could be injurious to plants. When calcium arsenate is present, no water-soluble copper is liberated because of the presence of free calcium, which, in every case tested, kept the pH of the mixture well above pH 5.5. Such is not true in the case of water-soluble arsenic. Different calcium arsenates produced varying results. As a general rule, when dealing with the same calcium arsenate, the lower the pH, the higher the water-soluble arsenic. Above pH 10.0, very little water-soluble arsenic is liberated. However, the character of the arsenate itself, rather than either the diluent or copper used, will determine the safety of the dust mixture. Limited studies with certain organic materials added to dust mixtures indicate that the above generalizations do not always hold.

Soil Fauna in Relation to the Pit Scab of Potatoes. GRANOVSKY, A. A., AND A. M. PETERSON. Further field studies of soil microfauna, habitually found in lesions of pit scab of potato (*Actinomyces scabies*) show it is not normally responsible for scab infection. About 50 per cent of pit-scab lesions were found infested with soil fauna, predominately mites, annelids, and nematodes. Other pits are free from animal life. Only a single record of what appears to be *Pnyxia scabiei* is known from the State in the past year. This insect is thought in other States to be responsible for pit scab. The high incidence of the disease and the scarcity of the insect requires a different interpretation of the problem. The maggots of Mycetophilidae are rarely found in the pits. Those found, are either *Sciara pauciseta, S. nitidicollis* and species closely related to *Forcipomyia pilosa*. More frequently encountered are several minute species of Collembola, belonging to *Sminthuridae, Isotomidae* and *Entomobryidae*. The most common inhabitants of pit scab lesions are mites—*Rhizoglyphus hyacinthi, Rh. phylloxerae* and species of *Macrochelus, Cheyletus* and *Uropodidae*. Most of these are scavengers or have predacious habits. Next in abundance are the young stages of annelid worms, which feed in the lesions and often completely clean the pits of decaying organic matter. Pit scab is the result of interaction of a physiologic race of *Actinomyces scabies*, secondary bacterial decay, and mycetophagous soil microfauna.

A Study of the Control of the Yellow Dwarf Disease of Potatoes. HANSING, E. D. Yellow dwarf can be most effectively and satisfactorily controlled in New York State by the use of potato varieties that escape infection under conditions favoring a moderate to high spread of the disease in the field. In several replicated experiments the varieties Arran Banner, Chippewa, Golden, Houma, Jubel, Katahdin, Sebago, and Warba had less than 2 per cent infected plants. The percentages of infected plants for Russet Burbank was 2 to 11, for Rural 3 to 29, and for Green Mountain 6 to 53. The percentages of infected plants for 20 U. S. D. A. potato seedling varieties ranged from 0 to 72. The incidence of yellow dwarf was partially reduced by such factors as location of the potato field; date of planting; use of shielding crops; roguing current-season infected plants; early harvesting; and the selection of potatoes for seed from the middle of the field. Application of dusts and spray, such as pyrethrum and celite, sulphur, or Bordeaux mixture, also reduced the amount of infection. Factors of little or no value in the control of the disease were: application of commercial fertilizer, roguing tuber-borne infected plants, and selection of large tubers for seed.

New Suscepts of the Potato Yellow-dwarf Virus. HANSING, E. D. The following species of plants belonging to 3 different families were found to be suscepts of the yellow-dwarf virus: Crassulaceae—*Kalanchoe diagremontiana*, Cruciferae—*Barbarea vulgaris* and *Capsella bursa-pastoris* and Leguminosae—*Medicago lupulina*. *K. diagremontiana* is an ornamental species introduced from Madagascar. The other 3 species are fairly common weeds in western New York, where epiphytotics of yellow dwarf occur. The following new varieties of known susceptible species also have been found to be suscepts of the virus: *Trifolium pratense* var. Mammoth Red and *Trifolium repens* var. English Wild White and var. Kent Wild White.

Biological Control of the Mealy Bug (Pseudococcus spp.). HARRAR, J. G., AND J. J. MCKELVEY, JR. A fungus, previously reported as a virulent, specific parasite of the mealy bug, has been investigated as a potential control of this greenhouse and orchard pest. Laboratory experiments under controlled temperature and moisture conditions were entirely successful in the destruction of mealy bug populations. Subsequently, similar experiments were carried out in greenhouses with equal success. Observations in apple orchards indicate possible high mortality of mealy bugs from natural infection and that the artificial addition of inoculum added may be beneficial. From data obtained, it is evident that the pathogen is rapid and positive in action under favorable conditions and will destroy mealy bugs in all stages of development (exclusive of the egg stage). Under unfavorable growth conditions, the fungus produces highly resistant compound sclerotia that may remain viable several months. These sclerotia are responsible for the persistance of the parasite in the field and greenhouse. The name *Endosclerotium pseudococcia* is suggested for the pathogen.

The Behavior of Endothia parasitica on Chestnut Trees in California. HARRIS, M. R. Three chestnut orchards, located near Stockton, San Joaquin County, California, have been found infected by the chestnut blight fungus (*Endothia parasitica*). Two of the orchards have been observed since 1934 and a third since 1938. The disease is not known to exist elsewhere in the State, although chestnuts are widely planted both as shade and orchard trees. The origin of the disease is not known, but the fungus probably was brought in on cions secured in Eastern States. Observations on every infected tree found over a period of years failed to show any evidence of the perfect stage of the fungus, but pycnospores have been noted on a number of the trees. Lesions of the fungus have been found less often on the limbs and trunks above ground line as at ground line or below it. Contrary to the experience with the disease in Eastern States, the fungus has been very largely spread by pycnospores carried by irrigation water. There is no evidence of spread by air, birds, or splashing rain. A few trees have been infected by contaminated pruning tools. Control of the disease is being carried on by inspecting the orchards twice a year and destroying infected trees. Present indications are that it may eventually be eradicated.

The Relation of Vitamin B_1 to Crown-gall Development. HENRY, BERCH W., A. J. RIKER, AND B. M. DUGGAR. Some relations of vitamin B_1 to crown-gall development on tomato have been studied with the Phycomyces assay. Vitamin B_1 accumulated in almost maximum concentration at inoculation points within 1 week after treatment. The B_1 concentration in galls remained fairly constant from 3 to 5 weeks after inoculation—during the period of rapid increase in size. The B_1 concentration in galls, approached that in the growing tip of the host plant, was somewhat greater than that in mature leaves, and was much greater than that in mature stems of either inoculated or control plants. Temperatures above and below the maximum for gall formation had no effect on the B_1 concentration in inoculated plants. Galls produced by a partly attenuated culture of *Phytomonas tumefaciens* contained as high a concentration of B_1 as did the galls produced by the virulent culture. The bacterial cells of the partly attenuated culture contained as much vitamin B_1 as the cells of the virulent culture. Thus, vitamin B_1 may help in the initiation of crown gall, but it does not seem to have a causal role in gall development beyond that of any necessary food or growth factor.

Improved Control of Alternaria solani (Early Blight) on Tomatoes by Controlling Flea Beetles. HEUBERGER, JOHN W. An experiment was designed to determine the effect of derris on the protective value of several copper compounds (Bordeaux, tribasic copper sulphate, copper oxychloride, yellow cuprous oxide, and red cuprous oxide). Six applications were made (June 30–Aug. 21). Flea beetles were abundant during June and July. Records on July 28 and Aug. 8 on corresponding plots receiving copper alone and copper-derris, as spray or dust, showed that the copper-derris plots had fewer beetle-feeding punctures, fewer blight lesions, and much less defoliation than the copper plots. Derris, by controlling beetles, improved blight control in two ways: (1) reduced the number of wounds (feeding punctures), which serve as infection courts, and (2) reduced dissemination of *Alternaria* spores. On Sept. 8 the copper-derris plots still had less defoliation, even though beetles disappeared by Aug. 1. Thus, the deleterious action of derris on copper effectiveness, recently reported, apparently was overbalanced by the beneficial effect of beetle control earlier in the season. The following schedule should give effective blight control in areas where beetles are present early: copper-derris while beetles are present, followed by copper alone at 10–14-day intervals after the beetles disappear.

Effect of Copper Content, Completeness of Admixture of Copper and Diluent, and Nature of Diluent on Field Performance of Copper Dusts. HEUBERGER, JOHN W. Copper compounds tested were copper oxychloride and red and yellow cuprous oxide; dust diluents were Pyrax ABB, Eastern Magnesia #23 Talc, Loomkill Talc, Bancroft Clay. The dust materials were tested also as sprays to eliminate such factors as fractionation in duster, flowability, denseness of dust cloud, settling rate, etc. Materials were tested on tomatoes for control of *Alternaria solani,* using a 6-application schedule (June 30–Aug. 21). Metallic copper content was 1.5–100 for sprays and 7 per cent for dusts. Using hand equipment, both were applied in similar fashion to deposit 3 lb. metallic copper per acre per application. Increasing copper content from 4 to 8 per cent increased control from 18 to 36 per cent; improving completeness of admixture of copper diluent in an attrition mill increased control from 16 per cent for original sample to 33 per cent; the nature of the diluent influenced effectiveness of copper *e.g.,* descending order of control with same copper, as sprays, was Bancroft Clay, Loomkill Talc, Eastern Magnesia Talc, Pyrax ABB, while the order, as dusts, was Loomkill Talc, Bancroft Clay, Eastern Magnesia Talc, Pyrax ABB. When applied in same fashion, similar materials used as sprays and dusts gave approximately equivalent control.

Transmission of Pierce's Disease of Grapevines with a Leaf Hopper. HEWITT, WM. B., N. W. FRAZIER, AND BYRON R. HOUSTON. The natural spread of Pierce's disease of grapevines indicates an insect vector. In 1939, 54 species of insects collected from vineyards, alfalfa, and natural cover were tested in a field plot as possible vectors. Ten of the 94 vines upon which one or more species of insects were caged and only one of 215 control plants developed Pierce's disease. Sixty insect species were similarly tested in 1940. Nine of these test plants developed disease, 6 out of 21 caged with leaf hoppers of the genus *Draeculacephala* and 3 out of 19 with the genus *Carneocephala,* and only 6 of the 506 control plants developed disease. Leaf hoppers of the genus *Draeculacephala* were further tested in 1941 under controlled insectary conditions. To date, 9 vines in these tests show leaf scorching and cane immaturity, typical fall symptoms of Pierce's disease. Field observations show a close correlation between the incidence of Pierce's disease and alfalfa dwarf. Insects that transmitted Pierce's disease in 1940, though fed on diseased grapes, had been collected from alfalfa fields known to have dwarf. In the 1941 tests the leaf hoppers that apparently transmitted the virus had been fed on alfalfa dwarf plants.

Host Response of Maize Seedlings to Pythium graminicolum. Ho, Wen-Chun, and James M. Koepper. During the past 3 years open pollinated varieties, inbreds, and single and double crosses of maize, grown in the greenhouse and field, varied widely in their response to *Pythium graminicolum.* In 14 varieties studied, Kossuth County Reliance and Stern Yellow Dent were most susceptible; Black Yellow Dent and Krug were resistant. Although the majority of the 15 inbreds tested were susceptible, Ldg (K), Black 349, Lancaster 289, Osterland 426 and Hy showed some resistance. Combinations of these less susceptible inbreds, in general, were the most resistant of all single crosses tested; Iodent 205 × Black 345 and Iodent 205 × Iodent 234 were very susceptible. The Iowa hybrids, Iowa 13, Iowa 931, and Iowa 939, seemed the most resistant of the 23 double crosses examined. The injury caused by *P. graminicolum* in combination with other pathogens was studied. Combinations with *Rhizoctonia solani, Helminthosporium sativum,* or *Diplodia zeae* increased the amount of injury. Combination with *Penicillium oxalicum* showed the same disease severity as *Pythium graminicolum* alone, but combinations with *Aspergillus niger* or *Trichoderma lignorum* caused less injury than *Pythium graminicolum* alone. In general, the symptoms from *P. graminicolum* and those from the active pathogens were distinct, but the destructive effect was additive. In combination with weak pathogens, symptoms of *P. graminicolum* prevailed, but the destructive effect was subtractive.

The Sorghum Root-and-stalk-rot Complex in Oklahoma. Hoffmaster, Donald E. Since 1939, a serious root-and-stalk-rot complex of sorghums has been under observation in Oklahoma. The disease complex may first appear when the plants are 3 or 4 inches tall, resulting in a seedling blight, and may continue to attack the surviving plants during the growing season, resulting in stunted plants with small, poorly developed heads. The symptoms of this disease complex closely resemble those of the Milo disease, but repeated isolations have failed to yield *Pythium arrhenomanes.* Instead, isolates of *Fusarium,* Bacteria, *Sclerotium bataticola, Helminthosporium* and *Pythium* (listed in order of prevalence) have been secured. Greenhouse and laboratory tests indicate that *Pythium* (not *arrhenomanes*) and *Sclerotium bataticola* are capable of causing seedling blight. The ability of *Sclerotium bataticola* to cause seedling blight is of especial interest because of the frequent occurrence of the fungus in stems of mature plants affected with charcoal rot. This is a disease frequently observed in plants subject to adverse environmental conditions.

Transgressive Inheritance of Pathogenicity Factors in Hybrids between Two Races of Tilletia tritici. Holton, C. S. Evidence of transgressive inheritance of factors for pathogenicity has been obtained from studies with hybrids between races 8 and 9 of *Tilletia tritici.* These races differ in that Hussar is susceptible to T-8 and resistant to T-9, while Hohenheimer is resistant to T-8 and susceptible to T-9. Hussar × Hohenheimer selection, C.I. 10068–1, is highly resistant to both of these races and all other known races of the bunt fungi, while Hybrid 128 is susceptible to all races. However, 4 hybrids between T-8 and T-9 prove capable of infecting all of these varieties and one other hybrid was pathogenic to Hybrid 128 only. The infection percentages on C.I. 10068–1 ranged from 29 to 56, thus indicating a rather high degree of susceptibility to the race hybrids. Furthermore those hybrids capable of infecting all of the above varieties were more virulent on Hohenheimer than T-9 and less virulent on Hussar than T-8. Apparently, therefore, in these hybrids between T-8 and T-9, transgressive inheritance of pathogenicity factors resulted in the production of biotypes possessing pathogenic properties, not only distinctly different from those of the parent races, but different from those of any other known race. (Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Washington Agricultural Experiment Station.)

The Effects of Mustard Oils on Germination of the Resting Spores of Plasmodiophora brassicae. Hooker, W. J. The effects of two mustard oils (allyl isothiocyanate and beta phenyl ethyl isothiocyanate) upon spore germination of *P. brassicae* were studied under laboratory conditions with partly purified suspensions of spores and various concentrations of oil. The extent of germination was determined by counting the number of zoospores within a field of known volume at daily intervals. Although the exact concentrations at which germination was prevented varied with different spore lots, both oils were consistently effective at 80 p.p.m. and sometimes at as low concentrations as 10 p.p.m. of allyl isothiocyanate and 5 p.p.m. of beta phenyl ethyl isothiocyanate. Concentrations of both oils below the toxic level were found capable of definitely stimulating spore germination. This occurred in each of the 8 experiments carried out with allyl isothiocyanate and with 2 of the 5 experiments involving the phenyl ethyl oil. Stimulation was greater in the 2 cases with phenyl ethyl isothiocyanate than in similar concentrations of the allyl oil prepared and observed at the same time. These observations suggest that a toxic substance present in the host may have a stimulatory action upon the pathogen at certain low concentrations.

Comparing Fungicides at Dosages for Equal Control. HORSFALL, JAMES G., AND ALBERT E. DIAMOND. While making a quantitative study of fungicide performance, it developed that comparison through (1) dosage-for-equal-control was more sensitive and more informative than (2) control-for-equal-dosage. Dosage for equal control can always be obtained by interpolation or extrapolation on the straight line obtained from the logarithmic-probability plotting of data. Control for equal dosage has a low order of sensitivity because the control scale is limited by a ceiling of 100 per cent, whereas the dosage scale is unlimited. Red and yellow copper oxide dusts applied to muskmelons against *Macrosporium cucumerinum* in 1941 are useful illustrations because the slopes of the curves are parallel. The ratio between the two on the control scale for equal dosages of 9 per cent metallic copper in the dust was 1.12, but this ratio was magnified to 1.77 on the dosage scale for equal control of 80 per cent. A more serious limitation of (2) is that the ratio in performance of the two materials changes along the control scale, rising to 1.24 with equal dosages of 4 per cent. Finally (*vide* Diamond accompanying abstract) the occasional inversion in rankings of materials may be detected in the data obtained in making a comparison at dosage for equal control.

Leaf-hopper Transmission of the Alfalfa Dwarf Virus. HOUSTON, BYRON R., N. W. FRAZIER, AND WM. B. HEWITT. The dwarf disease of alfalfa has recently been found prevalent in the southern portion of the San Joaquin Valley, California, where it is now an important factor in the thinning of alfalfa stands. In 1940 healthy plants growing in large cages infested by mixed species of insects collected in diseased fields resulted in 63 per cent dwarf diseased plants. Transmission tests also were made with individual species of plant-feeding insects collected in diseased fields. The only cases of transmission were in 2 of 3 tests with a leaf hopper of the genus *Draeculacephala.* A later trial with 100 individuals of this species caged on dwarf plants and subsequently transferred to 30 healthy plants resulted in 93 per cent disease. The virus was then transmitted from these diseased plants to healthy plants by grafting. Nine transmissions were later obtained with leafhoppers of the genus *Carneocephala.* In the summer of 1941, insectary inoculations were made involving several species of insects and several hundred alfalfa plants. To date 19 transmissions of alfalfa dwarf have been obtained with leaf hoppers of the genus *Draeculacephala* and no control plants have shown symptoms of the disease.

The Effect of Various Organic and Inorganic Compounds on the Growth of Sclerotinia sclerotiorum. HOYMAN, WM. G. Forty-four organic and inorganic compounds were tested in the laboratory to determine which would inhibit the growth of *Sclerotinia sclerotiorum.* When soluble, each compound was added to the medium in concentrations of 1: 1,000, 1: 10,000 and 1: 100,000. Lest certain transformations and losses might result from autoclaving the chemicals employed in this experiment were not thus treated. Malachite green, 3,5-dinitro-o-cresol, mercuric chloride, and 2,4-dintrophenol inhibited the growth of the organism at the lowest concentration (1: 100,000). These 4 chemicals were tested further at concentrations of 1: 200,000, 1: 400,000 and 1: 800,000 in order to determine which was the most toxic. Malachite green and 3,5-dinitro-o-cresol proved to be the most effective in inhibiting the growth of *Sclerotinia sclerotiorum.*

Breeding Cantaloupes for Resistance to Downy Mildew and Other Diseases and Pests. IVANOFF, S. S. Some progress has been made in breeding shipping-type cantaloupes for resistance, mainly to the downy mildew (*Peronoplasmopara cubensis*). Sources of resistance to downy mildew have been selected inbreds derived from the varieties Cuban Castillian and Rocky Dew (green flesh). Sources for commercial shipping qualities have been the varieties Arizona Nugget, Seed Breeders, and Powdery Mildew Resistant California 45. Some cantaloupe lines thus far obtained in the F_4, F_5 and F_6 generations have shown shipping qualities combined with varied resistance to downy mildew, powdery mildew (*Erysiphe cichoracearum*), the melon aphid (*Aphis gossypii*), and to damage caused on the leaves by larvae of *Diaphania* spp.

The Nature of Eggplant "Yellows." IVANOFF, S. S. This serious virosis of eggplants in South Texas, characterized by mottling, spotting, and yellowing of the leaves, has been found to be sap-transmissible. Its natural vector is still unknown. The disease has been transmitted to cucumber by rubbing with infectious juice, producing leaf mottling. It has been induced on eggplant by rubbing with juice obtained from cantaloupe plants grown in the field and exhibiting a type of mosaic symptoms. Efforts to transmit the disease from eggplants to tomatoes, peppers, potato, and other plants have thus far failed. Transmission from eggplant to eggplant has been accomplished also by grafting and by needle puncturing the stem through a drop of plant sap containing the virus. The incubation period varies from 8 to 21 days, apparently depending on the season, rate of plant growth, and source of inoculum. The infectivity of the virus seems to be seriously affected by drying. The virus appears to be closely related to the cucumber group of viruses.

A New Species of Elsinoë on Capulín Cherry (Prunus capuli). JENKINS, ANNA E. A new species of *Elsinoë* is described. This produces bright leaf spots on capulín cherry, closely related to wild black cherry (*Prunus serotina*). The type locality of the *Elsinoë* is the nursery of the Agricultural Experiment Station, Caracas, Venezuela, where the fungus was discovered in January, 1940, by M. F. Barrus and A. S. Müller, who forwarded specimens to the writer for study. The infected tree had been brought from Mexico. According to the literature Mexico is the country of origin of this much esteemed fruit tree, and the cherry was mentioned by the earliest Spanish visitors to the country. The perfect stage of the *Elsinoë* is present in abundance on the specimen mentioned, and the imperfect stage (*Sphaceloma*), on a subsequent gathering (March, 1941) by Müller. The existence of *Sphaceloma* on plum (*P. domestica*) was shown by the writer in 1932, through Garbowski's report of *Hadrotrichum populi* on this host growing on the peninsula Crimea (South Russia), or in its vicinity. Specimens are not available, however, as was learned from correspondence with Garbowski a number of years ago. During the past few years a hitherto unreported *Sphaceloma* on choke cherry (*P. virginiana*) was discovered in Canada and the United States by A. A. Bitancourt and the writer.

The Influence of Temperature and Moisture on the Development of the Intermediate Loose Smut of Barley. JOSEPHSON, L. M. Controlled experiments were conducted in the greenhouse at Madison, Wisconsin, to determine (1) the effect of constant soil temperature and soil moisture on the development of smut, and (2) the effect of change of soil temperature during the growth of the host on the incidence of barley smut caused by *Ustilago medians* (*U. nigra*). Soil temperatures of 5° and 30° C. were generally unfavorable, 10° and 25° C. more favorable, and 15° and 20° C. optimum for smut development. Air temperature did not appear to influence smut development when soil temperature was constant. Soil moisture did not give consistent results as a factor influencing smut development. Changes from the low to the optimum or high soil temperature after plant emergence resulted in more smut than maintenance of constant low temperature during the growth of the plants. Similar results occurred in the change from the high to the optimum soil temperature. However, changes from high to low and from optimum to either high or low soil temperatures decreased smut. The effect of temperature changes after the plants had reached the second or third leaf-stage was less pronounced.

Physiologic Races in the Fungus Causing the Intermediate Loose Smut of Barley. JOSEPHSON, L. M. Approximately 100 collections of *Ustilago medians* (*U. nigra*) from the North Central States were studied for their pathogenicity on 17 varieties of spring barley, critical tests having been made on 9 varieties. By tests covering a period of 5 years, 8 physiologic races of the fungus were differentiated. These races can be differentiated on the basis of their pathogenicity on the following varieties: Excelsior, C.I. 1248; Himalaya, C.I. 2448; Manchuria, O.A.C. 21, C.I. 1470; Wisconsin Barbless, C.I. 5105; Lion, C.I. 923; and Hannchen, C.I. 531. Odessa, C.I. 934, is susceptible to all 8 races. Race 2 was found in Minnesota only, races 4 and 5 in North Dakota only, race 7 in Illinois only, while races 1, 3, and 8 were widely distributed in the States from which collections were obtained. Race 6 was obtained from New York State, from which no other collections were received. Races 3, 4, and 5 are classified on the basis of small differences in pathogenicity, which make it desirable to further check these races under different environmental conditions.

The Compatibility of Fruit-drop Sprays and Other Common Spray Materials. KADOW K. J., AND S. L. HOPPERSTEAD. Sprays to prevent the premature dropping of apples are rapidly gaining general usage. When used on early varieties, it is often desirable to apply them along with other spray materials. Naphthaleneacetic acid (Parmone) was applied to Williams at 5 p.p.m. and to Delicious and McIntosh at 10 p.p.m. in combination with Black leaf 155, 3 lb.; Phenothiazine (Micronized), 2 lb.; Genicide, 1½ lb. + Genifilm A, 3 oz. + kerosene, 1½ pt. + Genifilm B, 2 oz.; Bordeaux 1–3–100 + lead arsenate, 2 lb.; lime, 20 lb. + aluminum sulphate, 3 lb.; and ground Derris (5% Rotenone), 3 lb. + Grasselli spreader sticker, 3 oz. In addition to the above combinations, the sodium salt of naphthaleneacetic acid (App-L-Set) was used at ¼ lb. with and without aluminum sulphate, 3 lb. + lime, 20 lb. Likewise, Fruitone, which is naphthaleneacetic acid + an amide of it, was also used (½ lb.) with and without aluminum sulphate, 3 lb. + lime, 20 lb. The results obtained indicate that naphthaleneacetic acid is compatible with all combinations except those containing lime. When lime was added the effectiveness of all three fruit-drop materials was reduced from 25 to 50 per cent. Lime, used as a spray ingredient earlier in the season, did not reduce the effectiveness of fruit-drop sprays, applied to the same trees later on. (Cooperative study with Grasselli Chemical Department, E. I. du Pont de Nemours Co., Wilmington, Delaware.)

The Production of Spores of Diplodia zeae in Culture. KENT, G. C. The production and germination of the spores of *Diplodia zeae* formed on the host or in culture has been found to vary greatly with the environmental conditions. The production in culture of spores of the most uniformly high germinability was secured by growing *Diplodia zeae* on oatmeal-extract agar solidified in a thin layer over the sides and bottom of an Erlenmeyer flask. The inoculated flask was incubated in a moist chamber and exposed to light and to a temperature of 20° C. After 6–10 weeks spores so produced were suspended in distilled water, washed twice and then suspended in the germinating medium, 2 per cent dextrose, 1 per cent starch, and carrot decoction being equally favorable under most conditions. Germination under the stated conditions occurred in 13–16 hours and was equally high, 95–100 per cent, whether or not the drop was covered by a cover slip. The percentage germination was lower if the spores were from cultures less than 6 weeks or more than 10 weeks of age.

Nitrogen Sources Utilized by Some Bacterial Plant Pathogens. KENT, G. C. The sources of nitrogen utilized by the peritrichous bacterial plant pathogens were studied as a possible adjunct to their classification. A test of usage was considered as positive only if the bacteria survived after 5 successive transfers at 48-hour intervals in liquid medium containing nitrogen in the test substance only. By this criterion, and with glycerol as a carbohydrate source, it was found that cultures designated as *Bacillus carotovorus, B. phytophthorus,* and *B. aroidae* could utilize nitrogen in the organic or inorganic form, whereas *B. amylovorus, B. tracheiphilus,* and *B. salicis* could utilize only complex organic forms of nitrogen. Of 2 cultures designated as *B. lathyri* one could utilize nitrogen as nitrate or ammonia, whereas the other could utilize only ammonia-nitrogen, in addition to the organic-nitrogen forms.

Reaction of Varieties and Selections of Oats to Pseudomonas coronafaciens. KINGSOLVER, C. H. Epiphytotics of halo blight occurred in Iowa in 1940 and 1941. The varieties Hancock, Marion, and Boone were more heavily infected than Fulghum, Gopher, Columbia, and Albion. Data obtained from breeding nurseries at Ames and Kanawha, Iowa, confirmed these field reactions. Most selections from the cross Markton × Rainbow were heavily infected. Selections of Victoria × Richland showed moderate amounts of infection; a few were only slightly infected. Most selections of Anthony × Bond showed moderate amounts of infection, however, the range was greater than in the Victoria × Richland selections. Almost all selections of D69 × Bond were outstanding in their relative freedom from infection. This was of interest because selections from this cross were resistant to most races of both rusts and both smuts. Certain of these selections will be increased in 1942 for possible distribution to Iowa farmers. Most selections from crosses involving Mutica Ukraina, (Victoria × Richland) × Columbia, Gopher × Boone and (Bond × Anthony) × Boone showed very heavy infection. Selections of (Iogold × Bond) × Boone showed consistently a moderate amount of infection. In *Avena byzantina,* Bond, Fulghum, and Victoria were outstanding in their freedom from infection. Preliminary greenhouse trials have substantiated field data. (Cooperative investigations of Botany and Plant Pathology Section, Iowa Agricultural Experiment Station, and Bureau of Plant Industry, U. S. Departmen tof Agriculture.)

Resistance in South American Lycopersicon Species to Early Blight and Septoria Blight. LOCKE, S. B. Application of a laboratory test to approximately 50 selections embracing 5 South American *Lycopersicon* species revealed in *L. hirsutum* resistance to *Septoria lycopersici* and *Alternaria solani,* the causal agents of Septoria blight and early blight, respectively. The laboratory test also showed the F_1 hybrids of the cross *L. esculentum* × *L. hirsutum* and the resistant parent equally resistant to Septoria blight, but intermediate with respect to early blight. Defoliation and leaf-spotting data, obtained in the field in 1941 under severe attacks by *Septoria lycopersici,* are in agreement with those from the laboratory tests. A field test for early-blight resistance was not obtained. Field data also indicated an approximate 1 to 1 segregation in the first backcross to the susceptible parent with respect to Septoria-blight resistance. While these data are not enough to establish the mode of inheritance, they do suggest that resistance to Septoria blight may be associated with a single, dominant genetic factor.

Biotypes within Puccinia graminis tritici, Race 15. LOEGERING, W. Q., AND E. C. STAKMAN. It has been known for some time that there may be biotypes within some physiologic races of *Puccinia graminis tritici.* Race 15 appeared especially suitable for study because collections of this race have fallen into two groups, designated for convenience 15A and 15B. Race 15A has been found in Japan and the United States. Race

15B has been found commonly in South America and is not uncommon in the United States. The latter is somewhat more pathogenic to some of the differentials than the former. Inoculations were made with representatives of both groups on several rust-resistant varieties of wheat and emmer. Rival, a newly recommended rust-resistant spring wheat, is resistant to 15A but completely susceptible to 15B, both in the seedling and adult-plant stages; hence it can be used as a differential. It is probable that the addition of other new varieties to the group of differentials may make possible a finer analysis of other physiologic races also. Practically, it is important, in testing for resistance, to inoculate new varieties with representative collections of races unless their composition is well known. (Cooperative investigations, U. S. Department of Agriculture and the Minnesota Agricultural Experiment Station.)

The Response of Some Field Crops on Soils Treated with Chlorpicrin. McLAUGHLIN, J. HARVEY, AND I. E. MELHUS. Duplicate soil-fumigation experiments were conducted on black, heavy Clarion loam and on light, sandy Buckner soil. These soils were treated with chlorpicrin in September, 1940, at the rate of 480 lb. per acre. Crops planted were oats, wheat, flax, barley, alfalfa, and sugar beets. Seedling-stand counts showed important differences in favor of treated over untreated soil. Isolations from seedling roots yielded fewer pathogens from treated than from untreated soil. The green and dry weights of the harvested crops showed pronounced differences in favor of treated over untreated soil with one exception: a planting of alfalfa on Clarion loam soil. Grain yields for the small grains and flax grown in treated soil varied from slightly greater to 5 times as great as the yield for those grown in untreated soil. The sugar beets grown in treated soil were 3 to 10 times the size of the beets in the untreated soil.

Witches' Broom of Alfalfa in Washington. MENZIES, J. D., AND F. D. HEALD. A diseased condition of alfalfa plants, characterized by the production of an abnormally large number of dwarfed, spindly shoots, has been reported occasionally in the northwestern States since 1924. It appears to be very similar to the destructive witches' broom virosis of alfalfa in Australia, but, in this country, has been considered to be of minor importance. Within the last 5 years, however, witches' broom has become epiphytotic in the Methow Valley of Washington, rendering 3- and 4-year-old alfalfa stands unproductive; in some cases it has completely discouraged reseeding to alfalfa. It is not uncommon to find fields wherein over 70 per cent of the plants are affected. Data suggest that this disease is due to a virus. Transmission attempts were made in 1940–41 using mechanical methods, grafting, and possible insect vectors. Limited variety trials show that the wilt-resistant varieties Ladak, Turkistan, and Hardistan are among the most susceptible to witches' broom, and no variety tested has shown marked resistance.

Physiologic Races of Ustilago tritici. MOORE, M. B. In investigations covering 6 years, at least 5 distinct physiologic races of *Ustilago tritici* have been distinguished by the relative ability of collections from different sources to cause infection on certain bread wheats and durums. In general, collections cause abundant smut in only one or the other group of wheats, although some can attack certain varieties in both groups. Most field collections have behaved like single races and have remained relatively constant over a period of years. In at least one case, however, there was a tendency for the virulence of a collection to decrease after successive annual inoculations on the same variety. When certain races were mixed, it was possible to separate them again by inoculating appropriate differential varieties, even after the mixture had been grown 1 year on a host equally susceptible to the component races. What was apparently the same race has sometimes been obtained from geographic regions as widely separated as Texas and the spring wheat region of Minnesota and North Dakota.

Studies on the Performance of Fungicide Diluents, Their Base Exchange and Copper-adsorption Capacities. NIKITIN, A. A., AND E. G. ANDERSON. It was found that commercially available dust diluents vary in their chemical composition, base exchange capacity, and adsorption properties. The term "inert," adopted for these materials, is misleading, since actually they have a great influence upon the performance of the copper fungicide. The difference in their behavior toward the copper ion is due to the difference in their base exchange capacity and to the variation in their physical properties, such as adsorption. The variation in the base exchange and copper adsorption capacities of the dust ingredients is of particular interest. The adsorption capacity of talcs for copper approaches that of limestone, while that of clays is much less than that of talcs. The buffering action of diluents possessing high adsorption capacities for copper may be beneficial to crops most sensitive to copper injury. Less adsorptive materials may be suitable on resistant plants. Adsorption properties of diluents also may protect against leaching of soluble copper by rainfall. Spreaders, such as wheat flour, soya flour, or bentonite, change the adsorption capacity of the diluents. It was found that the adsorption capacity of talc was reduced by bentonite or flour.

Pathogenicity Studies of Rhizoctonia Isolates on Potatoes and Beans. PERSON, L. H. Rhizoctonia cultures were isolated from single sclerotia from potatoes from Nebraska, North Dakota, Maine, and Colorado. From 65 of these, tested on potato stems in sterilized soil, 23 were non-pathogenic, 27 only slightly pathogenic (producing only light, superficial lesions), 13 moderately pathogenic, and only 2 produced severe lesions. Two isolates from sugar beets and 2 from bean stems produced severe lesions on potato stems, while one isolate from bean and one from cauliflower produced moderate lesions. Of the 65 potato isolates tested on bean seedlings, 54 were non-pathogenic, 9 produced only slight superficial infection centers on 16 out of 267 seedlings, and 2 were only slightly pathogenic, producing small but definite lesions. The bean, sugar-beet, and cauliflower isolates produced moderate to severe lesions on the bean seedlings. In previous work (unpublished) 11 potato isolates in 6 different tests were found non-pathogenic on bean seedlings. This indicates that Rhizoctonia isolates obtained from potato tubers are not pathogenic on bean seedlings, and that lesions associated with potato stems are probably to a greater extent caused by soil-borne Rhizoctonia than by Rhizoctonia developing from the sclerotia borne on the seed potatoes.

Physical and Chemical Adaptation and Environmental "Carry Over" Effects in Ustilago zeae. PETTY, MILTON A. A constant haploid monosporidial line of *Ustilago zeae* was grown for 7 continuous cultural generations at about 21° C., at 32° C., and 35° C. Duplicates were exchanged among the temperatures. At 32° C. and at 35° C. there arose 13 mutants, 1 temporary variant that persisted for 2 generations, and 2 variants that did not persist beyond the generation of their origin. The constant and a variable haploid monosporidial line were grown as many as 9 cultural generations on synthetic media containing sodium arsenite, mercuric, ferric, and sodium chlorides whose concentrations were usually increased each generation. Cultures thus gradually adapted grew on a 0.558 per cent arsenic medium, but unadapted cultures failed to grow. Mutations occurred frequently on arsenic media in both lines. No definite results were obtained with either mercury or iron media, as the results were quite variable from generation to generation. Both lines of *U. zeae* grew faster after 3 generations on a 10.8 per cent sodium chloride medium than did first-generation cultures. This was apparently an osmotic adaptation. In conclusion, acceptable scientific proof was not obtained because the possibility of mutation could not be eliminated in the experiments.

The Reaction of Cantaloup Strains to Powdery Mildew in the Greenhouse and in the Field. PRYOR, DEAN E., AND THOMAS W. WHITAKER. Symptoms of cantaloupe powdery mildew were separated into 5 classes. Utilizing this classification, the problem of comparing symptoms in the greenhouse with those occurring in the field has been approached by two methods. (1) A comparison has been made of the symptoms in strains of cantaloupes artificially inoculated in the greenhouse with those of plants exposed to natural field infection. Of the 18 strains tested in the greenhouse, all except one highly resistant strain showed a 4 reaction (most severe). Infection on these same strains in the field varied with the planting date, being more severe in the last two plantings than in the first one. Except for the last planting, in which all the tolerant and susceptible strains gave a type-4 reaction, a majority of the tolerant strains were superior to the susceptible check. (2) Records of individual plants tested in the greenhouse, then transplanted to the field, indicate that if all macroscopic symptoms be taken into account, susceptible plants can be eliminated dependably in the greenhouse. These experiments have served as a basis for the development of a greenhouse technique that provides an accurate and practical method of judging the mildew resistance of cantaloupe without the time or expense involved in field trials.

The Influence of Vitamin B_1 on the Development of Cantaloupe Powdery Mildew. PRYOR, DEAN E. Of the two melon strains employed, the susceptible variety was readily attacked by race 2 of *Erysiphe cichoracearum*, while the resistant line developed necrotic spots following severe artificial inoculation. When thiamin chloride in various concentrations was added to soil in which diseased plants were growing, the number of colonies on the susceptible melons was increased 30 to 50 per cent, and necrosis on the resistant strain 110 to 180 per cent over the controls. The differences were statistically significant. When leaves from mildew-free susceptible plants were excised, inoculated, and maintained on a sucrose solution to which various amounts of thiamin were added, non-significant increases in mildew resulted. When leaves from mildew-free plants, grown in thiamin-treated soil, were excised, inoculated and maintained on the sucrose solution alone, those from plants previously watered with .01 p.p.m., thiamin solution produced barely significantly more mildew growth.

Experiments on the Yellows Disease of Sour Cherry (Prunus cerasus). Rasmussen, E. J., and Donald Cation. Cherry yellows was transmitted by budding from cherry to cherry, cherry to peach, peach to peach, and peach to cherry. Inoculations in 1939 on 6-year-old Montmorency cherry trees resulted in symptoms 2 years later, while 1940 inoculation of spring-planted Montmorency year-old nursery trees produced symptoms the following year. Control trees showed no symptoms. Three strains of cherry yellows have been differentiated by reactions on peach and Mahaleb cherry. Peach trees proved an excellent indexing medium for cherry yellows, showing symptoms the year following budding in all cases. On peach, die back, streak, and spot necrosis in the cortex and retarded foliation are characteristic of all strains of the virus. In addition, chlorotic mottling, leaf distortion, shortened internodes, and dwarfing of the tree result from 2 strains. One strain is milder on peach, another produces ring spot on Mahaleb leaves. Peach trees did not react to inoculations from normal cherry trees. Known varieties of peach, such as Hale and South Haven, reacted uniformly to a given strain of cherry yellows, while seedling peaches reacted variably. Eight soil treatments were ineffective in control. Cherry yellows reduced fruit yield by half in observed cases.

The Effect of Cotton-Seed Dusting on Emergence of Seedlings in Soil Infested with Rhizoctonia. Ray, W. Winfield. The effect on the emergence of cotton in soil heavily infested with *Rhizoctonia solani,* after treatment of the seed with various kinds of proprietary dusts, was determined in greenhouse experiments. All results obtained were analyzed by the analysis-of-variance-method. Some organic-mercury dusts increase more than do others the emergence of seedlings over the nondusted seed. Copper dusts are somewhat effective, but less so than the best of the mercury compounds. Spergon was the most effective of all those dusts lacking a heavy metal as the active ingredient. It was equal in effectiveness to the best organic mercury dust. Seeds dusted with the various agents tested gave a significantly greater emergence than nondusted seed in nearly every instance, but, so far as the final stand was concerned, dusted seed had little value as a protectant against *Rhizoctonia.*

Control of Diseases of Garden Roses. Rosen, H. R. During a 4-year period, red cuprous oxide dust mixture (4 per cent cuprous oxide, 2 per cent calcium arsenate, 10 per cent flour, and 84 per cent talc) applied once a week during the growing season, gave excellent control of black spot on susceptible hybrid teas, without injury to foliage under Arkansas conditions. Of numerous fungicides under investigation, it is the only one that gave such results. Breeding for disease resistance has resulted in a number of winter-hardy and mildew-resistant hybrids. Most of these hybrids are climbing types resulting from crosses between *Rosa setigera* derivatives and various bush hybrid teas. *R. setigera* appears to offer an excellent source of resistance to winter injury, powdery mildew, and drought and heat injury but not to black spot. Floral characters of its hybrids are diverse, some desirable, others not.

Crown-rust Infection of Oats as Related to Reduction in Hardiness and Yield. Rosen, H. R., and L. M. Weetman. Susceptible varieties of oats planted early in September, 1940, became infected with crown rust within 6 weeks, the infections averaging over 40 per cent of leaf areas. The same varieties planted in October showed few or no infections. Along with susceptible varieties, some 5,000 selections of oat hybrids, bred primarily for crown-rust resistance, were also planted early and late. With no opportunity for hardening-off prior to a severe November freeze, all plants of hardy varieties heavily infected with crown rust showed a large amount of frost injury, while plants of the same varieties with little or no crown-rust infection showed a correspondingly lower amount of frost injury. Hardy, crown-rust-resistant strains of both early and late plantings showed a minimum of such injury. The fall epidemic of crown rust on susceptible varieties resulted in a reduction in winter pasture and apparently in grain yields. In the absence of an epidemic in the spring of 1941, susceptible varieties planted late in the fall of 1940, which had escaped the fall epidemic, gave larger grain yields than the early fall plantings. Hardy crown-rust-resistant strains planted early or late in the fall gave exceptionally high yields.

The Influence of Temperature, Moisture, and Soil Reaction on Damping-off of Red Pine by Pythium and Rhizoctonia. Roth, L. F., and A. J. Riker. In Wisconsin forest nurseries *Pythium irregulare* and *Rhizoctonia solani* were the principal causal agents of damping-off. The predominance of one over the other sometimes occurred in different nurseries at the same time, and sometimes in the same nursery at different times. Since the activity of one or the other apparently depended on local conditions, investigations were made of the effects that some important environmental factors had on the development of disease. Greenhouse studies with seed of red pine, planted in Plainfield sand, showed that within limits the maximum damping-off from *Pythium* occurred below 24° C.

and that from *Rhizoctonia* above 24° C. Soil moistures somewhat less than 70 per cent moisture-holding capacity were favorable to *Rhizoctonia* and those more than 70 per cent were favorable to *Pythium*. Saturated air humidity favored *Rhizoctonia*, but did not influence *Pythium*. Within limits soil reactions that were more acid than pH 5.8 were favorable to *Rhizoctonia*, those more alkaline than pH 5.8 were favorable to *Pythium*. The red-pine seed grew well in soil between about 15° and 30° C., with medium moisture, and with a reaction between about pH 4.7 and 6.0. Damping-off was favored, within limits, when emergence and maturity were prolonged by the environment.

Loss of Sporulation in Cercospora. RYKER, T. C. Cultures of certain species of *Cercospora*, regardless of whether they originate from tissues of the host or from single spores, eventually produce feebly sporulating mycelial variants that overgrow the cultures. Very often, in transferring, the original types are lost. The original type, however, can be maintained by transferring from hyphal tips or from conidia. The most common variant is a white mycelial non-conidial form, which usually originates as a patch variant in old cultures. This white variant remains fixed. It has been found common to a number of species of *Cercospora*. From a study of 38 single hyphal-tip isolates of germinating conidia of *C. oryzae*, *C. beticola*, *C. apii*, and *C. nicotiana*, it has been determined that all produce these white mycelial variants, which are remarkably similar in culture. As the cells of the conidia are uninucleate, it is assumed that these variants are mutations. The loss of sporulation in cultures of these species of *Cercospora* is ordinarily due to the development of the non-conidial variants that overgrow the original cultures.

The Importance of Seed Transmission of Early Blight and Fusarium Wilt of Tomato. SAMSON, R. W., T. J. NUGENT, AND L. C. SHENBERGER. The possibility of important transmission of *Alternaria solani* and *Fusarium lycopersici* inside of seed from tomato fruits sufficiently free of decay for juice and purée manufacture was found to be extremely remote, even though the fruits were sorted from crops infected with early blight and fusarium wilt. No seeds internally infected with *A. solani* were found among 15,590 extracted commercially from such tomatoes. Only 2 were found among 5659 plated from seed lots from cull fruits and portions of fruits showing abundant early blight infection. Four of 400 seeds from selected decayed regions of infected fruits yielded pathogenic cultures. No internal infection by *F. lycopersici* was demonstrated in 20,207 seeds from commercial lots saved from seed-production fields containing wilt-infected plants. Colonies of *Fusarium* grew from 4 out of 3690 seeds plated from ripe, edible fruits taken from infected pedicels or stems. No *Fusarium* was secured from 675 seeds removed aseptically from fruits evidencing vascular invasion. The usual methods of surface disinfection with calcium or sodium hypochlorite and mercuric chloride were used for the most part in the plating of seed to determine internal infection.

Comparative Reactions of Single Crosses of Dent Maize to Diplodia zeae. SEMENIUK, G. Forty-nine single crosses of dent maize were tested for comparative reactions to *Diplodia zeae* in 1941 by a greenhouse seedling-infection method and a field stalk-inoculation method. Greenhouse tests were conducted in freshly steamed 2:1 field soil-sand mixture placed in 4-in. unglazed flower pots. *Diplodia zeae* soil-cornmeal inoculum and maize seeds were planted simultaneously. The inoculum was placed as an even layer, 5 g. per pot at the seed level in one group of pots, and 20 g. per pot at 2 cm. below the seed level in another group. The seedlings were examined for infection after 3 weeks. In field tests stalk inoculations were made with an aqueous spore suspension at the fourth internode above the ground level on August 12–13. On September 18–20 the development of *D. zeae* in the pith was measured after splitting the stalks. Significant differences in reaction of the single crosses were obtained in (1) the extent of mesocotyl and primary root necrosis in seedlings when the inoculum was placed at the seed level, (2) the extent of spread and rotting of the pith of stalks after inoculation, and (3) the number of dead stalks resulting from natural causes. No significant interrelationship was found between greenhouse and field data, whereas significant correlations were obtained in the field data.

Diplodia Epidemic in Conifer Seedbeds. SLAGG, C. M., AND ERNEST WRIGHT. Dead and dying seedlings of *Pinus nigra*, *Pseudotsuga taxifolia*, *Pinus ponderosa*, and *Pinus edulis* in seedbeds at a Federal nursery at Manhattan, Kans., were found infected with a fungus identified as *Diplodia pinea* (*Sphaeropsis ellisi*). The same fungus was found on 2- and 3-year-old stock of *Pinus sylvestris*, *P. nigra*, and *P. ponderosa* at the nursery, and on 10- to 50-year-old trees of *P. nigra*, *P. ponderosa*, and *P. sylvestris* on the campus of Kansas State College at Manhattan. In the seedbeds, injury was most severe on *Pinus nigra* and *Pseudotsuga taxifolia*, approximately 50 per cent of the seedlings being diseased or dead. These seedbeds had been sown with dry seed 5 months before the epidemic was observed and the young plants had not yet completed development of woody tissues.

Disease symptoms were reproduced in 5 days on similar seedlings of *P. nigra* from an uninfected nursery inoculated with mycelium of single-spore cultures of the fungus.

Cross Inoculations with Pythium arrhenomanes from Cereals and Grasses in the Northern Great Plains. SPRAGUE, RODERICK, AND R. E. ATKINSON. Forty of 600 isolates of *P. arrhenomanes*, selected to include those from 18 species of cereals and grasses collected at various locations in the Northern Great Plains, were parasitic on all hosts in cross inoculations in the greenhouse. Isolates from durum wheat, emmer, oats, rye, proso, *Agropyron cristatum, A. repens, Bromus inermis*, and *Elymus junceus* were particularly virulent on all hosts tested. Pre-emergence blight was most severe—often 100 per cent —on *Bouteloua gracilis, Oryzopsis hymenoides, Panicum miliaceum, P. virgatum, Phleum pratense, Setaria italica*, and *Stipa viridula*. Corn, wheat, sorghum, sudan grass, *A. cristatum, Bromus inermis*, and *E. junceus* showed less pre-emergence blight, but were invariably definitely stunted. In the case of *A. cristatum, B. inermis*, and *E. junceus*, injury was much greater when old, small, or moldy seed was used, seed strain causing more variation in root rot than the source of the fungus. (Cooperative investigations of the Divisions of Cereal Crops and Diseases, Forage Crops and Diseases, and Dry-Land Agriculture, Bureau of Plant Industry, and the Nursery Division, Soil Conservation Service, U. S. Department of Agriculture, and the North Dakota Agricultural Experiment Station.)

Recent Changes in Prevalence of Physiologic Races of Puccinia graminis tritici in the United States. STAKMAN, E. C., AND W. Q. LOEGERING. Race 56 of *Puccinia graminis tritici* increased in prevalence in the United States each year from 1930 to 1938, inclusive, and was the most prevalent race from 1934 to 1940, but it began to decrease in 1939 and in 1941 was surpassed by race 17, which had shown a tendency to increase gradually from 1930 to 1939 and then increased rapidly from 10 per cent of all isolates in 1939 to 34 per cent in 1940 and 52 per cent in 1941. If these trends continue, Thatcher wheat should retain its resistance in the spring wheat region, as it is highly resistant to race 56 and immune from race 17, which together comprised 84 per cent of all racial isolates in 1941; and, taking all races into consideration, it either is immune from or resistant to 94 per cent of those isolated. On the other hand, the commonly grown durums are resistant to only 37 per cent of the isolates obtained in 1941, as contrasted with about 70 per cent in 1938 before the decided increase of race 17, to which they are susceptible, and the decrease of race 56, to which they are resistant. It is not improbable, therefore, that stem rust may again become important in the durum wheat region. (Cooperative investigations, U. S. Department of Agriculture and the Minnesota Agricultural Experiment Station.)

Inactivating in Vivo the Virus of X-disease of Peach by Chemotherapy. STODDARD, E. M. Since 1935, 45 organic and inorganic chemicals have been used to treat living peach tissue infected with the virus of X-disease. These chemicals were applied chiefly by soaking 10 randomized diseased buds in water solutions of the chemicals. Nearly all the buds grew when budded into healthy seedlings. The most complete experiment was made in 1941. Eight of the 10 diseased buds, soaked in quinhydrone, failed to produce the disease as compared to 1 in the checks and none in some of the treatments. 8-hydroxyquinoline sulphate, hydroquinone, p-nitrophenol, Calcium 8-hydroxyquinolate, urea, sodium thiosulphate and some of their derivatives inactivated the virus in lesser degree, under like conditions, at the concentrations used. In a preliminary test, in 1940, buds soaked in urea, calcium 8-hydroxyquinolate, magnesium 8-hydroxyquinolate, o-nitrophenol, and sodium thiosulphate have failed to produce the disease to date. In these experiments most of the buds failing to produce the disease grew and developed normal healthy shoots. These data show that the virus of X-disease of peach can be inactivated *in vivo*, by chemicals, and that diseased tissues will recover and grow normally. They offer the hope that other viruses will respond similarly.

The Thread-blight Fungus, Corticium stevensii. TIMS, E. C. The thread-blight fungus, *Corticium stevensii*, is found on a number of host plants in Louisiana. Cultures obtained from sclerotia from several of these hosts have been quite similar in culture and in pathogenicity on fig leaves and twigs. Single-basidiospore cultures obtained from fig leaves also have been uniform in cultural characters, and in all cases similar to the cultures obtained from sclerotia or from spore masses. The monosporous cultures produced basidial mats with typical basidiospores when inoculated individually on fig leaves. Many of these cultures also produced typical hyphal threads and sclerotia on fig twigs when the leaves were inoculated. The constant similarity in all cultures also produced typical hyphal threads and sclerotia on fig twigs when the leaves were inoculated. The constant similarity in all cultures of this fungus, whether obtained from sclerotia, spore masses, or single basidiospores, and the uniform way in which the monosporous cultures produced normal fruiting structures on fig leaves indicates that *C. stevensii* is, apparently, a homothallic form.

Studies in the Classification of the Bacterial Plant Pathogens. WALDEE, E. L. Comparative studies of phytopathogenic bacteria having peritrichous flagella revealed the existence of at least 3 easily recognizable generic groups. The genus *Erwinia* in the restricted sense suggested in a previous abstract does not fit into any of the existing families of bacteria. It is proposed, therefore, that a new family in the order Eubacteriales be recognized with *Erwinia* designated as the type genus. The soft-rot bacteria were found to be more closely related to the coliform bacteria than to the members of the genus *Erwinia.* Because they constitute a distinct taxonomic unit it is proposed that the soft-rot bacteria be incorporated into a separate genus in the family containing the coliform bacteria. The yellow organisms, including *B. lathyri, B. ananas* and *Bact. stewartii,* are not yet well understood and occupy a doubtful position.

The Classification of the Cornstalk-rot Pathogen. WALDEE, E. L. Eleven isolates of the cornstalk-rot pathogen were studied comparatively together with more than 40 isolates of soft-rot bacteria and representative cultures of 4 species of coliform bacteria. The results of this study show that the cornstalk-rot pathogen is not a soft-rot organism, but that it is a member of the coliform group of bacteria, usually nonmotile, gram-negative, and rod-shape. Glucose, lactose, and cellobiose are fermented with production of acid and much gas (not less than 10 per cent and usually more than 50 per cent in Durham tubes). Nitrates are reduced to nitrites. The organism does not produce indol, is methyl red-negative, Voges-Proskauer positive, and grows well in Koser's citrate medium. No protopectinase is secreted. These and other reactions indicate that the organism is a member of Aerobacter section of the coliform bacteria.

Physiologic Races of Plasmodiophora brassicae. WALKER, J. C. Several varieties of turnip and rutabaga tested with various collections of *P. brassicae* from widely separated localities in the United States were found to remain completely free from development of clubs when grown in heavily infested soil. One of these, Purple Top Milan turnip, grown on naturally infested soil in 2 locations in England developed about 20 per cent diseased plants in each location. An English variety, White Stone, which showed 87 per cent diseased plants in an English test, failed to develop any clubbed roots with a representative American isolate. This is submitted as proof that definite physiologic races of *P. brassicae* exist. The tests in England were carried out by F. T. Bennett in the North of England and by N. C. Preston in Shropshire. (Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, and University of Wisconsin.)

Systemic Invasion of Cabbage by Plasmodiophora brassicae. WALKER, J. C. Under greenhouse conditions when cabbage seedlings are grown in soil infested with *P. brassicae* the pathogen, after infecting the root, may migrate through the cambium into the stem. There is relatively little cambial proliferation in the internodal regions above the third or fourth leaf. Dormant buds at the leaf scars, however, are stimulated to grow, and become invaded by the pathogen. They become malformed due to extreme hyperplasia. The organism may reach the growing point in young plants and cause extreme distortion of stem and leaves. When plants are inoculated at aboveground leaf nodes, the pathogen may migrate down the stem, leaving no evidence of proliferation in its path until the hypocotyl is reached, where a typical club is formed. There is evidence that the reaction of the host is influenced by the nutrient supplied to it. (Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, and University of Wisconsin.)

Virus Strains in Relation to Acquired Immunity from Curly Top in Tomato. WALLACE, J. M. Plants of cultivated varieties of tomato (*Lycopersicum esculentum*) very rarely recover and acquire an active immunity from curly top. However, they can be passively immunized by grafting with tobacco (*Nicotiana tabacum*) plants that have acquired an immunity. The clonal progeny of passively immunized tomato plants, when grown in the field under severe curly-top exposure, showed a high degree of protection, but occasional plants within an immunized clone developed severe curly-top exposure, showed a high degree of protection, but occasional plants within an immunized clone developed severe curly top. This led to a study of strains of the virus, which showed that tomato plants immunized against single virus strains were either unaffected, mildly affected, or quite severely affected, depending upon the strain of virus used for reinoculation. Healthy, non-immunized plants were severely affected by all of the virus strains used for immunization and reinoculation. This role of virus strains in the field of acquired immunity in plants parallels certain reactions in the animal virus field, where strains of a given virus differ antigenically, the immune-sera of the different strains not providing cross immunity. It is believed that these data furnish additional evidence that acquired immunity from curly top results from the production of some type of protective substances.

Genetic Studies in Oats of Resistance to Two Physiologic Races of Crown Rust. WEETMAN, L. M. Inheritance studies have been made of the types of resistance to crown rust found in 3 varieties of oats used as resistant parents in breeding work. These varieties are Bond (resistant to race 1, susceptible to race 45), Victoria (resistant to both races 1 and 45), and Mutica Ukraina (resistant to race 45 and to a portion of race 1). F_2 and F_3 data indicate that the Bond type of resistance to race 1 is conditioned by two dominant complementary genes. Victoria resistance to both races 1 and 45 appears to be largely determined by a single factor which is distinct from the Bond genes. F_2 field data indicate that the Mutica resistance to the major portion of race 1 is due to two dominant complementary genes as in Bond. These genes are probably allelomorphic to the Bond genes for resistance.

Further Studies on Pythium Injury of Oats. WELCH, AARON. Under Iowa conditions *Pythium debaryanum* and related species of *Pythium* have proved to be serious parasites on the roots of oat plants. From 1937 to 1941 these organisms were prevalent throughout the State and were isolated each year from oats growing in widely separated localities. Under controlled greenhouse conditions 218 varieties of oats (including winter, spring, and wild types) were grown in steamed soil artificially infested with *P. debaryanum.* Marked resistance was not observed in any one variety, but some varieties were more resistant than others. The effects of phosphorous, potash, nitrogen, manure, and a complete fertilizer on the ability of *P. debaryanum* to parasitize oat roots failed to indicate that any of the treatments were beneficial in reducing or controlling infection. Oats grown in plots treated with chlorpicrin, however, yielded 100 per cent more than the same varieties grown in non-treated fertilized plots.

Some Tentative Conclusions Resulting from Plot Analyses of Phomopsis-blighted Juniper Seedlings in Great Plains Nurseries during 1941. WRIGHT, ERNEST, AND C. M. SLAGG. Because of need for information on control of Phomopsis blight of junipers in broadcast sowings, the following tentative conclusions may be of interest. Contrary to current opinion, 1-year-old eastern red cedar (*Juniperus virginiana*) seedlings originating from Kansas-collected seed proved no more resistant to infection by *Phomopsis juniperovora* than those grown from Nebraska seed. Likewise, western red cedar (*J. scopulorum*), previously not sprayed because it was considered highly resistant to the disease, was so severely infected that spraying will be necessary in the future. Infection of juniper seedlings usually started at the margin of the seedbeds, eventually worked inward, and appeared to be directly associated with water splashing. Seedlings watered by overhead irrigation were, therefore, more severely blighted than those watered by ditch irrigation. Dense stands, presumably because they reduce water splashing, tended to retard rather than to increase the percentage of infection. Roguing of diseased plants was not significantly beneficial except in plots where infection was relatively light. To be most effective, the seedlings should be rogued as soon as they show the first symptoms of infection before sporulation begins. Of 7 sprays used, commercial Bordeaux (5–5–50) gave the most promise of controlling the disease.

Stimulatory and Toxic Effects of Copper Sprays on Powdery Mildews. YARWOOD, C. E. The dried deposit of 0.1 per cent Bordeaux ± 0.1 per cent cottonseed oil on glass slides was toxic to conidia of *Erysiphe polygoni* from red clover and bean in water, approximately neutral on dry slides at 100 per cent R.H., and stimulatory at 90 per cent R.H. Copper sulphate of Bordeaux added to sucrose agar stimulated the germination of bean powdery-mildew conidia, but inhibited the growth of contaminating fungi. On inoculation, beans sprayed with 0.1 per cent Bordeaux frequently showed more mildew development on the upper surface of primary leaves than did unsprayed plants. This stimulatory effect of Bordeaux was greatest when light was inhibitory to mildew development, though the nature of the inhibition by light is not understood. Many fungicides, including Bordeaux and lime sulphur, were more effective as eradicant sprays for powdery mildew on beans, cucumbers, and cantaloupes, and for rust on bean and sunflower on plants held in moist chambers for several hours after spraying than on plants dried soon after spraying. Most fungicides tested were more effective as eradicant than as protective sprays for powdery mildews.

Fungicidal Value of Bordeaux Mixtures Prepared from Diluted and Concentrated Stock Solutions. YARWOOD, C. E. Concentrated bluestone Bordeaux prepared by adding 13 per cent $Ca(OH)_2$ to 10 per cent $CuSO_4 \cdot 5H_2O$ and then adding the required amount of water, was compared with diluted bluestone Bordeaux prepared by adding 13 per cent

$Ca(OH)_2$ to $CuSO_4 \cdot 5H_2O$, which had first been diluted to spray strength, as protective sprays for onion downy mildew, hop downy mildew, cucumber downy mildew, cucumber powdery mildew, bean powdery mildew, and bean rust, and as eradicant fungicides for bean powdery mildew and cucumber powdery mildew in greenhouse tests under conditions of heavy artificial inoculation. In 66 paired comparisons there was little difference in the fungicidal value of the diluted bluestone Bordeaux and concentrated bluestone Bordeaux. Mixtures containing 0.03 to 0.3 per cent bluestone gave marked control of all diseases, except as protective sprays for bean mildew. Only in the case of bean rust was the diluted bluestone Bordeaux markedly superior to the concentrated bluestone Bordeaux. The addition of certain spray supplements increased the protective value of most sprays but did not affect the relative merit of diluted bluestone and concentrated bluestone Bordeaux.

Toxin Formation and Chemotherapy in Relation to Dutch Elm Disease. ZENTMYER, GEORGE A. *Ceratostomella ulmi* was found to produce in culture a toxin that wilts plant cuttings and induces symptoms similar to those of the Dutch elm disease when injected into elms. This suggests that the fungus produces in the tree a similar toxin, responsible for disease symptoms. In chemical treatment of 850 small (3–6-ft.-tall) American elms, 5 of 25 organic chemicals gave promising results in preventing or retarding progress of disease. Injecting hydroquinone, benzoic acid, or p-nitrophenol, 1 week before inoculation with *C. ulmi,* greatly reduced the percentage of trees showing disease symptoms as compared with water-injected controls. When 50 trees were injected with 8-hydroxyquinoline sulphate and simultaneously inoculated, the appearance of symptoms was significantly retarded for 2½ weeks, as compared with 50 noninjected controls. When inoculated trees were injected with benzoic acid, hydroquinone, or 8-hydroxyquinoline benzoate at the onset of wilting, the further advance of wilting was markedly retarded as compared with water-injected controls. Retardation of disease by injection of these chemicals offers hope that the Dutch elm disease and similar vascular diseases may ultimately be controlled by chemotherapy. Results indicate that once the disease becomes well established the possibilities of checking its advance by chemotherapy are considerably diminished.

ABSTRACTS PERTAINING TO DEMONSTRATIONS

Western Red Rot in Ponderosa Pine in Arizona and New Mexico. ANDREWS, STUART R., AND LAKE S. GILL. Western red rot, caused by *Polyporus ellisianus,* is responsible for a major proportion of the 25 per cent defect frequently found in mature and overmature ponderosa pine stands in the Southwest. Studies during 1938 and 1939 to determine its importance in immature stands indicated that, since western red rot enters trees almost exclusively through dead branches, initial infection is but slightly contingent on age and suppression, but is highly dependent on the branch habit of trees. In the 41–100-year age class, of the trees with only dead branches less than 1.1 inches in basal diameter, 8.0 per cent had infected branches, as compared with 33.8 per cent of those having one or more branches larger than that size. Although infection also was correlated with tree age, size, and stand density, this may be attributed to the high correlation between these factors and branch habit. Age alone was found highly correlated with the progress of decay in the heartwood of the trunk. Studies made during the second cutting of a tract in northern Arizona indicated that the proportions of gross volume lost because of western red rot amounted to 0.8 per cent in trees 121–150 years old, 4.6 per cent in those 151–180 years old, and 10.3 per cent in those 241–270 years old. It would appear that while western-red-rot losses may be somewhat less severe in second-growth than in old-growth stands, they can be further reduced if stands are kept dense enough to inhibit large-branch formation, pruned at an early age, and harvested before they are overmature.

Cross-Inoculations with Fusarium-wilt Organisms. ARMSTRONG, G. M., B. S. HAWKINS, AND C. C. BENNETT. Fusarium-wilt organisms were obtained from the following plants: cotton, okra, watermelon, sweet potato, tobacco (3 sources), tomato, mimosa (*Albizzia julibrissin*) and coffee weed (*Cassia tora*). Inoculations of all hosts, except mimosa, were made either in soil or water culture with at least 3 of the organisms. Records were made of external symptoms of wilt, internal darkening, and recovery of the fungus by plating. The isolates from cotton, okra, coffee weed, and tobacco in South Carolina and Kentucky are apparently the same. All cause wilting in the first 3 hosts and in a susceptible Burley tobacco but not in a resistant Burley or Gold Dollar (flue-cured variety). A Maryland tobacco isolate caused wilting of all the above tobacco varieties as well as Maryland Mammoth but did not affect cotton or okra. The cotton fungus, however, caused wilting of Maryland Mammoth tobacco. The sweet-potato fungus did not cause wilting of cotton, nor did the cotton fungus cause wilting of sweet potato. The sweet-potato fungus, however, caused wilting of a susceptible Burley tobacco. The tomato fungus failed to cause wilting in cotton, okra, and watermelon and the watermelon fungus failed to cause wilting in sweet potato and tomato.

A Method of Inoculation for Barley Stripe. ARNY, D. C., AND H. L. SHANDS. The stripe reaction of barley varieties and selections may be had from the following test: Sterile water is introduced into a 7- to 12-day-old test-tube slant culture of *Helminthosporium gramineum* and the mycelium scraped off in fine aggregations. Two cc. of this suspension are then added to 125 ml. Ehrlenmeyer flasks previously prepared by adding 15 g. wheat and 15 cc. water, and autoclaving 45 minutes. When fungous growth on the wheat is 5 days old, 25 to 100 kernels of each selection being tested are treated with alcohol to remove surface contaminants, rinsed in water, and placed in one of the flasks. Kernels in contact with inoculum are incubated 4 days at room temperature (20 to 24° C.). Lower temperatures produce more infection if time be adjusted. Flasks are shaken daily to prevent clumping caused by mycelial and root growth. Larger flasks may be used to accommodate more kernels. The entire contents of the flasks are planted in soil. A susceptible variety, such as Oderbrucker, usually has 80 per cent or more infection. Ordinarily about 50 per cent of the kernels planted in the field produce plants, while over 80 per cent may be expected in the greenhouse.

Bacterial Necrosis of the Giant Cactus. BROWN, J. G., LAKE S. GILL, PAUL C. LIGHTLE, AND DON M. HEEP. Work on bacterial necrosis of the giant cactus (*Carnegiea gigantea*), reported by the senior author and co-workers at the last Philadelphia Meeting, A. P. S., has been cooperatively organized by the Arizona Agricultural Experiment Station and the Bureau of Plant Industry, U. S. Department of Agriculture. Survey of large plots in the Saguaro National Monument have been completed in which the incidence of necrosis has been determined and mapped. Activities in attempted eradication or control of the disease include sanitary measures and surgery, in cases of early stages of infection. Necrosis in the Monument and in other giant-cactus forests in Arizona is now very active. Species of *Opuntia* thus far inoculated with the proved cause of necrosis in *Carnegiea* have shown no symptoms of the disease.

Response of Diseased Maple Trees to Chemotherapy and Fertilization. CAROSELLI, N. E., AND F. L. HOWARD. Average twig elongation has shown highly significant differences when maple trees afflicted with bleeding canker were given the following treatments: (1) Injected with antidotal chemicals and subsequently fed by the soil crow-bar method; (2) injected and not fed; (3) fed and not injected, and (4) left untreated. Five mature trees were used for each treatment and the twig growth measured for each of the 6 years 1936 to 1941, inclusive. Ten samples of twigs taken at random from each of the top, middle, and lower sections of each tree were measured. No significant difference was found to exist within treatments. Twig elongation (1) was about uniform for the healthy trees; (2) gradually decreased about one-half during the 6 years for the untreated diseased trees; (3) decreased to the minimum for diseased trees fed but not injected; (4) increased somewhat during the current year and sharply the following year for diseased trees that were injected; and (5) was greater during the year injected and increased most markedly in the next year for both injected and fed. Improvements in injection technique will be demonstrated.

Masking of Wheat Leaf-rust Infections by High Temperatures. CHESTER, K. STARR. Wheat seedlings inoculated with leaf rust (*Puccinia triticina*) and then incubated at temperatures above or below the optimum for rust production, frequently fail to manifest infection, leading to erroneous conclusions regarding host susceptibility or race identity. In an attempt to understand this temperature-conditioned inhibition of rust reaction, seedlings of the 8 wheat varieties used in leaf-rust race differentiation were inoculated with races 5, 19, 20, and 34 and incubated at various temperatures between 39° and 77° F. Highly susceptible varieties, when incubated at 55° F., showed in numerous cases no infection if incubated for 24 hours at higher temperatures and then exposed to optimum temperatures for long periods, indicating that at the higher temperatures no infection occurred. When similarly inoculated plants were exposed to 55° F. for the first 24 hours and then transferred to higher temperatures for 10 days or longer, no infection was apparent, but rust pustules promptly appeared whenever such plants were returned to 55° F., indicating that infection occurred during the first 24 hours at 55° F. but became latent at higher temperatures.

Behavior of Dwarf Mistletoes of Western Conifers in Relation to Control. GILL, LAKE S., AND STUART R. ANDREWS. The dwarf mistletoes (*Arceuthobium* spp.) are so abundant and harmful in parts of the West that there is much practical interest in the effect of old infected trees left standing after logging on the infection of the young stand.

In ponderosa pine, in northern Arizona, where a heavily infected stand had been logged 30 years earlier, data were obtained in 1939, on an area of 60 acres, on the behavior of *A. vaginata* forma *cryptopodum* in the understory of seedlings and saplings. The majority of these had not become established until some time after the original cutting; infection in them was unusually low and for the most part restricted to stands within a radius of 50 feet of infective overstory trees. The parasite had had no significant influence on the height of seedlings, largely because almost all infections were of recent origin, no infection having occurred prior to 1926. Nevertheless, since 70 per cent of the infections occurred on the main stems of seedlings, mistletoe will eventually cause deformity or serious reduction in growth, if not death. In addition, about one-half of those in branches were so close to the main stems as to indicate extension of the endophytic system into the trunks.

Symptom Expression in Psorosis of Citrus as Related to Kind of Inoculum. FAWCETT, H. S., AND L. C. COCHRAN. Sweet-orange trees developed from buds of trees infected with the virus of psorosis A (*Citrivir psorosis* var. *vulgare*) require an incubation period of 5 or more years before bark lesions appear. In some trees this time may be 20 years; the average is between 12 and 16 years. Older trees inoculated with buds taken from twigs not showing bark lesions on psorosis-afflicted trees also require 5 or more years for bark symptoms to appear. If, however, patches of live bark, cut with a cork borer, from the center of scaling lesions, on a Psorosis A-affected tree are transplanted to trunks of sweet-orange seedlings, bark scaling may begin in less than 5 months. Trees inoculated similarly from normal bark 3 to 6 inches away from the lesions have shown no bark lesions after 3 years. Some indication was obtained that patches taken from the middle showed a shorter incubation period than those from the advancing edge of the lesion. Inoculation with the virus of Psorosis B (*Citrivir psorosis* var. *anulatum*) gave corresponding but shorter incubation periods. This method not only opens a new approach to the character of the psorosis virus, but suggests a method of greatly shortening the incubation period.

Fumigation of Potting Soils. GODFREY, G. H. Soils completely free from nematodes and from damping-off and root-rot fungi, and practically free from weeds, can be kept available for use by the simple and relatively inexpensive means of fumigation. Chloropicrin or methyl bromide at 4 ml. per cu. ft. have been found highly effective for killing soil fungi, as well as nematodes (*Heterodera mariona* and *Pratylenchus pratensis*). Carbon bisulphide and ethylene dichloride at 10 ml. per cu. ft. have killed nematode infestations satisfactorily, but are not so reliable against fungi. Recent tests with methyl bromide have indicated that, while it is difficult to handle because of its low boiling point, it is highly promising for soil fumigation because of its apparent ability to penetrate undecayed nematode galls. Galvanized cans or gas-tight boxes are suitable for soil containers for fumigation. In all cases means must be provided for sealing a gas-tight cover. The soil should be loose and free from excessive wetness, and fumigation done while temperature is high. Applicators are available at much lower cost than heat-sterilizing equipment.

The Relation of Certain Weevils to Root Rot and Basal Stem Rot of Cereals and Grasses. HANSON, E. W., AND H. E. MILLIRON. Certain weevils infesting Gramineae are important in the development of root rot and basal stem rot of cereals and grasses in the Northern Great Plains. Several species of these weevils are found in this area, and it is known that some of them attack many hosts. The widely distributed *Calendra parvula* is an important species recorded in our investigations as attacking wheat, timothy, bluegrass and crested wheatgrass. Most of the insect injury is confined to the lower internodes of the culms. Very severe rotting is consistently associated with such infestation. The work of this weevil provides avenues of entrance for fungi and bacteria and promotes the development of rots in the basal parts of the plants. The infested internodes are filled with frass, resulting from larval feeding, which is an excellent medium for the rapid increase of microorganisms. The dissemination of these rot-inducing organisms appears to be facilitated by such feeding and the movement of the larvae inside the plants. (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Division of Plant Pathology and Botany, and Entomology and Economic Zoology of the Minnesota Agricultural Experiment Station.)

Antidoting the Toxins of Plant Diseases. HORSFALL, JAMES G., AND GEORGE A. ZENTMYER. It is known that many of the pathogenic effects in plant diseases are produced by toxins, and that even the parasitism of many organisms may be possible only because excreted substances precede the invader into the host, killing the tissue and preparing the way for further advance. Research in 1940 and 1941 indicated that toxins of

Dutch elm disease and Verticillosis of eggplant and maple may be weakened, possibly eliminated, by internal chemotherapy with the following compounds and their derivatives containing basic nitrogen: 8-hydroxyquinoline sulphate, urea, dihydrochloride of diaminobenzene, malachite green, and probably ammonia. Since the effects of several root diseases are known to be reduced by ammonia, apparently through some non-fungicidal mechanism, these results suggest that such root diseases are toxin-induced and that the basic nitrogen in ammonia antidotes the toxin. These results also suggest an additional approach to chemical control of plant disease. By and large, chemicals are applied externally to the host, *i.e.*, aimed at killing potential parasites. Chemicals are applied internally here, more with the aim of interfering with the mechanism of pathogenesis than of killing the parasite.

Life Activities and Control of Phytophthora citrophthora. KLOTZ, L. J., AND H. S. FAWCETT. Hyphae grown on alfalfa stems produce abundant functional sporangia when placed in well-aerated water. Sudden lowering of temperature causes mature sporangia to discharge zoospores. A swimming zoospore takes a spiral course and revolves continuously on its long axis. The cilium, extended forward, propels by means of a corkscrew motion; the other trails, rudderlike. Either can assume the function of the other, making it unnecessary to about face to reverse direction. Germination produces 1 to 5 germ tubes. Diplanetism is frequently exhibited. Under favorable temperature and moisture any organ (bark, fibrous roots, leaves, flowers, and fruit) can be infected at any point, no wound being necessary. Hyphae invade inter- and intracellularly. Control measures are based on zoospore sensitivity to heat and cuprion. Grove protection is afforded by copper sprays, good drainage, aeration, and sunlight. Sunlight raises exposed bark temperature 15° to 25° F. above air temperature and, in summer, kills invading fungus. Rind of lemons can also be heated to such lethal temperatures by immersion in water at 118° to 120° F. In storage, contact infection by aerial hyphae can be suppressed by prompt removal of rots, lowering humidity to 75 to 80 per cent, and maintaining a concentration of 1 to 2 mg. of NCl_3 gas per cu. ft. for 4 hours at weekly intervals.

Production of Disease-resistant Sorghums. MELCHERS, L. E., F. A. WAGNER, AND A. E. LOWE. The Southern Great Plains area produces several million acres of milo and milo hybrids for grain. They are susceptible to the milo disease. The Kansas Agricultural Experiment Station, in cooperation with the Division of Cereal Crops and Diseases, U. S. D. A., has studied the cause and control of this malady. The exhibit illustrates the various steps followed in selecting resistant plants in the field and greenhouse, how these selections are further tested to eliminate accidental "escapes," segregates, or varietal mixtures, and how disease reaction and agronomic character may be determined in infested soils. The use of greenhouse methods demonstrates how selection for resistance may be accomplished and how these methods are used in testing certified seed of resistant strains of milo. Photographs show how county agents and growers may have soil from questionable fields tested for the presence of the disease. Living plant material of resistant selections produced at the Kansas Agricultural Experiment Station will be shown.

Seed Treatment of Good Seed Corn. REDDY, C. S., AND W. N. RICE. Seed treatment of good seed corn is beneficial when adverse conditions prevail at planting time. Treatment is of little or no value when the seed is planted in moist or dry soil that remains warm for several days. When the seed is planted in somewhat dry soil that remains cold for several days (in demonstration 7 days at 10° C.), seed treatment is of value in obtaining better field stands. The value of seed treatment increases under cold soil conditions with increases in soil moisture. The limit of value is reached when stands from treated seed are entirely satisfactory and stands from non-treated seed are so poor as to necessitate replanting. The demonstration shows the effect of seed treatment using a commercial organic mercury dust, New Improved Semesan Jr., and an experimental organic dust, Spergonex. Seed lots vary in degree of response to temperature and moisture. This degree of response is the basis for the seed-laboratory "cold-test" in which a special tensiometer is used to insure the same moisture factor for each test.

Persistence of Cotton-root-rot Sclerotia Following Certain Cropping Practices. ROGERS, C. H., AND HERBERT RICH. Cotton-root-rot (*Phymatotrichum omnivorum*) sclerotia have been found at depths to 8 ft. in the Texas blackland soils and appear to be one of the most important factors in survival of the fungus. Most of these sclerotia occur in the upper 3 ft., approximately half the total being found at depths of 12 to 24 in. Large quantities of viable sclerotia were found in soil after 12 years of continuous cropping with cereals, which are non-susceptible, and after the same period of clean fallow. Sclerotia may thus retain their viability and infect susceptible plants after lying dormant in the soil for long periods of years. Sclerotial populations varied from a few thousand to 10,000,000 or more per acre foot of soil.

A Comparative Study of Four Species of Rhizoctonia. RYKER, T. C., AND BEATRICE EXNER. Four rhizoctonia diseases in the South can be distinguished on the basis of symptoms produced on various hosts and the appearance of the isolates on culture media. They are the banded sclerotial disease of Bermuda grass, rice, and sugar cane caused by *Hypochnus sasakii;* web blight of beans and figs and other plants caused by *R. microsclerotia;* an undescribed blight of figs caused by a *Corticium* sp., and the various root and stem rots caused by *R. solani.* Studies of the Corticium stages of the 4 have shown no distinct differences either in size and shape of basidiospores or in appearance of basidial mats. Tissue isolates of diseased material have shown very little variation and it has been possible to classify such isolates satisfactorily in the 4 groups. On the other hand, while some cultures from single basidiospores of *H. sasakii, R. microsclerotia,* and the *Corticium* from fig have been similar to the tissue cultures, most of them have been so different that they could not be placed in any one of the 4 groups. Single basidiospore isolates of *R. solani,* however, while showing some variation, could all be classified as *R. solani.* The results suggest the possibility that the 3 former fungi are hybrid forms and that the variation is due to segregation.

Pythium Injury to Flax. SCHLICK, R. W. Flax develops poorly following sugar beets. Stands are poor and the developing plants lack the vigor and color of healthy flax plants. In searching for the cause of this condition a species of *Pythium* (*P. debaryanum* group) was obtained in a high percentage of the isolations made from seedlings. In many cases, before the seedlings had emerged, the radicle was discolored and the cotyledons rotted within the seed coat. In less severe cases only the tip of the radicle showed a reddish-tan discoloration, and these seedlings emerged. In the former case it appeared that this species of *Pythium* can cause a direct general necrosis. Isolates from more mature plants in the spring and early summer were predominately *Pythium* sp., sometimes associated with *Rhizoctonia* sp., *Fusarium* sp., *Helminthosporium* sp., etc. The lateral and tap roots showed a light reddish-tan to brick-red discoloration. A few small lateral lesions on the tap root showed similar discolorations. These attacks were restricted to the cortical tissue.

Placement of Diplodia zeae Inoculum in the Soil in Relation to Infection of Maize Seedlings in Greenhouse Pot Experiments. SEMENIUK, G. *Diplodia zeae* soil-cornmeal inoculum was placed as a uniform layer below, at and above the seed level. Inoculum in 5–20 g. amounts and seeds were planted at the same time in potted, freshly steamed sand and soil-sand mixture. Seedlings were examined after 3 weeks at 68–75° F. Severe mesocotyl and primary root necrosis initiated at the point of their juncture was obtained from inoculum placed at or above the seed level. Only few small primary root lesions were obtained from inoculum placed 2 or 4 cm. below the seed. Mean air temperatures of approximately 65, 75, and 85° F. yielded similar infections from inoculum at and below the seed. Sand yielded fewer root lesions from inoculum placed below the seed than did a soil-sand mixture. Soaking seed 10 minutes in a 1: 10,000 ethyl mercury phosphate solution markedly reduced infection from inoculum placed at and above the seed, but did not influence root infections from inoculum below. Isolated necrotic lesions on the mesocotyl were rarely observed. The washing down of spores from the inoculum above the seed was suggested. *Diplodia zeae* was unable to grow sufficiently in steamed soil-sand pot experiments from below the seed to establish infection at the seed level.

Acquired Immunity from Curly Top in Tobacco and Tomato. WALLACE, J. M. A summary of the results of the study of acquired immunity from curly-top virosis is presented and illustrated. Special attention is given to the phases of this investigation in which the plant-virus relations are similar to immunological phenomena in the field of animal viruses.

Wilt-resistant Tomatoes with New Genetic Characters. YOUNG, P. A. Cuttings of *Lycopersicon chilense* × *L. esculentum,* received from F. O. Holmes, bore flowers but no fruit. Pollen from them was back-crossed onto *L. esculentum.* Progeny plants showed intermediate inheritance of finely dissected leaflets; plants with prominently dissected leaflets were sterile. Fruits on the other plants were 1 to 2 cm. in diameter, and yellow or orange; few plants had red fruits, which indicated unusual inheritance of fruit color. Striped fruits appeared on one plant of the variety Michigan State. Stripes and corky pits appeared in the fruit peel of most of the progeny plants of the 2nd and 3rd generations. In a field of Rutgers tomatoes, one plant bore lobed, nearly hollow fruits, 7 cm. in diameter. This fruit character bred true through 3 generations. Apparently resulting from X-ray-induced mutations, one tomato selection had yellowish bordered leaflets. Another selection had white flowers. The white-flower character was transmitted to hybrids nearly immune from Fusarium wilt. One plant of the variety Prairiana bore sticky fruits associated with light-green leaflets and hairy stems. These characters bred true through 3 generations.

THE EFFECT OF CERTAIN CHEMICALS, SOME OF WHICH PRODUCE CHROMOSOME DOUBLING, ON PLANT TUMORS

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INTRODUCTION

Since 1937, colchicine has been used extensively by geneticists to attempt the improvement of such flowers, vegetables, and fruits as will respond to its stimulation by the production of new and desirable qualities. It is extracted largely from the plant *Colchicum autumnale* L., the autumn crocus, abundant in Asia Minor. Early Greek physicians used it as a remedy for gout; even today it is used to a limited extent in the treatment of arthritis. It was a physician treating a tumorous patient for gout who noticed and recorded the fact that colchicine delayed the advance of the tumor. Discovery of this record years afterwards induced some animal research workers to experiment with the alkaloid; they were followed by students of animal cancer, geneticists, and plant pathologists.

Colchicine, in very weak solution, is used by geneticists in the attempt to produce new varieties of plants. The alkaloid induces a doubling of the number of chromosomes in the nucleus. A short exposure to a weak solution limits the amount of doubling that takes place. A strong solution or a long exposure to a weak one would defeat the result hoped for. With a short exposure to a weak solution, cell division continues in the regular manner; and, after division, the new-formed cells contain the increased number of chromosomes. The changed nucleus with its added number of chromosomes has its effect on the plant, and some new and distinct type of variation may be the result. One of the methods for producing new varieties of plants is to dip young growing stems in a 0.2 per cent solution of the alkaloid.

In experimenting on the effect of colchicine on bacterial plant tumors, it was found that it could not only inhibit their growth but also kill them. The simplest and most direct way to introduce the alkaloid was to brush a 2 per cent aqueous solution on the surface of the tumor with a camel-hair brush. When temperature and moisture conditions were especially favorable for penetration, a 1 per cent, or even 0.5 per cent, solution would bring about the desired result. A 3 or 4 per cent solution proved no more effective than the 2 per cent solution, nor did death follow its application any more readily. Brushing the tumors with colchicine solutions of these concentrations had no apparent effect on the other parts of the plants; they grew, blossomed, and produced seed quite normally.

Having studied the relation of colchicine to polyploidy in various plants, my colleague, Haig Dermen, became interested and made a study of the nuclei of cells of colchicine-treated plant tumors before death took place. He found that, at mitosis, the chromosomes continued to divide repeatedly.

There was no indication of spindle formation or migration of chromosomes to the poles or of cross walls forming to produce daughter cells. The separate chromosome divisions massed together in one huge nucleus with irregular lobes (2, 3, 4). He estimated that the normal number of chromosomes—48 for the French marigold, *Tagetes patula* L.—might divide until there could be as many as 24,500 chromosomes holding together in one irregular nucleus, the cell increasing in size correspondingly. As this was an abnormal condition the plant could not overcome, death of the tumor cells occurred, and, eventually, death of the entire tumor followed.

The death of bacterial plant tumors subsequent to brushing them with a 2 per cent solution of colchicine was reported in 1939 (1, 2). With plant tumors as with healthy plant tissue, the cells must be young and actively dividing if the number of chromosomes is to be increased by the application of colchicine. It is apparently the stimulation to excessive doubling without cell division that destroys the balanced metabolism of the cells. This condition is attributable to the many chromosomes massed together in the huge lobed nucleus; and it follows that the tumor cells increase in size correspondingly. Because the plant cannot adjust itself to this abnormal condition, the tumor cells become shrunken and dry and the tumor itself dies.

This rather exhaustive introduction is submitted to make clear the connection between the earlier work on plant tumors with a polyploidizing chemical (colchicine) and that here presented, recording experiments in treating plant bacterial tumors with other chemicals claimed to be chromosome-doubling (6, 7, 8, 10, 13). In other words, if these other chromosome-doubling chemicals produce polyploidy for geneticists, would they not also induce an excessive number of chromosomes in tumor cells and thereby kill the tumors, as did one application of a 2 per cent solution of colchicine?

SUBSTANCES OTHER THAN COLCHICINE THAT CAUSE CHROMOSOME DOUBLING

The effect of some, perhaps all, of the other chromosome-doubling substances is similar to that induced by colchicine on wheat, rye, and other seedlings (11, 14). The roots of the treated seedlings thicken, and at the ends there are tumor-like swellings the cells of which show abnormal mitosis (5, 6, 9, 12, 14).

The chromosome-doubling substances used in these experiments were as follows: Acenaphthene, a-methylnaphthalene, a-nitronaphthalene, 3–5-dibromopyridine (15), and apiole (7). A commercial preparation known as "Santomerse," a few drops in water, was used as an emulsifying agent.

Experiments with Acenaphthene

When acenaphthene was mixed with lanolin and brushed on 12 French marigold and on 16 Paris daisy (*Chrysanthemum frutescens* L.) tumors 1 cm. or less in diameter, the treated tumors were all alive and growing at the end of a month and were the same size as the controls.

Acenaphthene, in a filtered, saturated water solution, was brushed for 2 successive days on 55 bacterial tumors, less than 1 cm. in diameter, on French marigold. Twenty days later the tumors were of the same size and appearance as those of the controls. Leaf galls of French marigold and stem tumors of Guinea-gold, a variety of African marigold (*Tagetes erecta* L.), also were brushed with a filtered, saturated water solution of acenaphthene. No change in the tumors followed.

Because it was thought that failure of acenaphthene to cause injury might be due to its slight solubility in water, other solvents were tried.

A number of solvents of acenaphthene were tested on plant tumors before applying that chemical to learn the possible reaction of the galls to the solvent. Several had a killing effect in 24 to 48 hours, and were discarded. Among these were morphalin, tetralin, and dimethyl phthalate. Those found most satisfactory in not affecting the tumors, or the stems in the vicinity of the tumors, were chlorinated naphthalene and dioxan.

Although not a solvent, glycerin was mixed with acenaphthene at the rate of 5 cc. to ½ g. of acenaphthene. This paste-like mixture was spread thickly on the galls, in the hope that there would be a slow but definite penetration of the acenaphthalene into the cells. No injury occurred, nor was there any change in the bacterial tumors. Growth continued as in the controls.

Chlorinated naphthalene proved to be a solvent of acenaphthene, but there was neither killing nor inhibition of growth subsequent to application of a solution to tumors of various ages. Either the acenaphthene did not penetrate far enough into the tissue or the solvent neutralized its action. Some other factor may have been working, for a reaction was noted in the very young galls. Stimulation of growth of tumors occurred in 28 marigold and 21 brushed daisy galls, and these were definitely larger than those brushed with chlorinated naphthalene for controls or the galls left unbrushed for other controls. The stimulation occurred slowly, was not excessive, and no collapse of tumor cells followed. Stimulation was not observed in tumors that were 27 or more days old before treatment.

Besides increase in size over the control tumors, the galls brushed with acenaphthene chlorinated naphthalene continued both to grow and live longer than did the controls. Tests made with both daisy and marigold tumors in summer and fall gave like results.

Five, 10, 15 per cent, and full-strength solutions of dioxan[1] showed no harmful effect when brushed on both young and old daisy and marigold tumors. Because the tumors did not discolor and continued to grow vigorously, it was considered safe to use dioxan as a solvent.

Marigold galls of different ages were brushed with different percentages of acenaphthene dissolved in dioxan. It was thought that some combination of age of tumor and strength of acenaphthene might result in sufficient penetration of the chemical to stimulate rapid chromosome divisions, so that a

[1] Dioxan is the trade name for diethylene oxide.

collapse of young growing cells would finally occur. The action and effect of colchicine would thus be simulated. However, this did not happen with marigold tumors, regardless of their age. Solutions of acenaphthene in dioxan were used up to 10 per cent. In this experiment, 164 marigold tumors, 7, 11, 12, 14, 15, 16, 21, 22, and 34 days old, respectively, were brushed from 1 to 4 times. There were 91 controls for comparison.

Death did not occur when marigold tumors were brushed 4 successive days with a 3 per cent acenaphthene solution and the plants were kept in a moist chamber to prevent surface drying. In the moist chamber there was some penetration as indicated by shrinking and darkening of the tumors, but there were enough tumor cells alive and functioning to allow *Bacterium tumefaciens* to get a new start. This was shown by the development of outgrowths at the edges of the shrunken and darkened tumors. These rejuvenated tumors eventually became as large as the controls. Thirty-one treated marigold galls and 47 controls were studied under moist-chamber conditions.

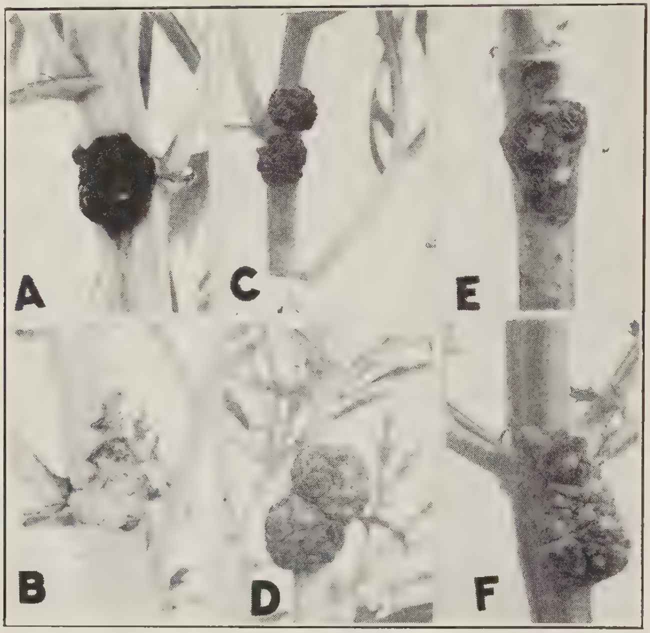

FIG. 1. A. Tumor on Paris daisy produced by inoculating with *Bacterium tumefaciens*, September 3, 1940; brushed with 3 per cent acenaphthene in dioxan, October 4, 1940; photographed October 10, 1940. B. Control of A. C. Paris daisy inoculated with *Bact. tumefaciens*, May 7, 1940; brushed with a-methylnaphthalene 50 per cent, June 14, 1940; Photographed, June 17, 1940. D. Control of C. E. Guinea gold marigold inoculated April 22, 1940; brushed with n-heptyl aldehyde 20 per cent May 17, 1940. Gall dead in three days, photographed June 7, 1940. F. Control of E. The brushed galls were dead when photographed, or died a few days later. All × 1.

Death occurred with daisy tumors, brushed with 3 per cent acenaphthene dissolved in dioxan, if the tumors were 36 or more days old and still growing. Tumors 31 days old or less did not all succumb. Some darkened, some died (Fig. 1, A; control B), and the growth of others was inhibited. Out of 44 treated daisy tumors of different ages, 24 died.

A saturated water solution of the slightly soluble acenaphthene was used for watering marigold seedlings, later inoculated with *Bacterium tumefaciens* to learn whether or not the intake of this chemical would inhibit tumor production. Twenty-five marigold seedlings 2 to 2½ in. tall, grown in 3-in. pots, were watered daily for 10 days, each plant receiving 20–25 cc. of the solution. Sometimes crystals of acenaphthene were visible on the surface of the soil, but apparently without seedling injury. On the 10th day the 25 plants were inoculated, also 10 control plants. In 5 days the swellings at the points inoculated on the treated plants were larger than those on the control plants, a stimulation probably due to the chemical. The leaves were larger and had a broader horizontal spread in the treated plants than in the controls. This difference, however, lasted no longer than a week, after which the controls and treated plants appeared the same.

Four marigold and 4 daisy plants, 13 and 17 days old, respectively, bearing galls and growing in 6-in. pots were also watered with the acenaphthene-saturated water solution. The marigold galls were 5–7 mm. and the daisy galls 4–8 mm. in diameter. Each plant received 150 cc. of the solution per day for 10 days. Neither stimulation nor inhibition of the tumors on the treated plants was noted in comparison to the controls of the same age.

Haig Dermen examined free-hand sections of young daisy galls that had been brushed 3 times with 3 per cent acenaphthene dissolved in dioxan. The galls were first brushed when 16 days old and were examined 10 days later. In the interim they had received 2 other brushings with the same chemical. Dermen's statement is as follows: "Judging from 2 free-hand sections of young daisy galls, one treated with acenaphthene and the other untreated, the effect of acenaphthene appears to be comparable to that of weak colchicine (0.01 per cent solution) brushed on young marigold tumors." As it required a 1 to 2 per cent solution of colchicine to induce definite polyploidy in young daisy and marigold tumors, and ultimately kill them, the effect of the 0.01 per cent solution was slight; increase of nuclear and cell size occurred near the periphery (3) or in small areas (4).

Experiments with a-methylnaphthalene

A-methylnaphthalene in a saturated water solution, also a-nitronaphthalene and 3–5-dibromopyridine in saturated water solutions were all brushed twice on 12 French marigold and 16 daisy tumors, as had been done with acenaphthene. Lanolin, mixed with each of these chemicals, also was brushed on other marigold and daisy tumors. All results were negative.

Since a saturated water solution of a-methylnaphthalene had no effect when brushed on marigold or daisy tumors, the straight chemical and a 50 per cent emulsion were tested. Figure 1, C, shows a 38-day-old daisy tumor brushed once with a 50 per cent emulsion. The photograph was made 3 days after brushing. The tumor was dead in 5 days. A control tumor of the same age is shown in figure 1, D.

The full strength of a-methylnaphthalene was brushed once on marigold tumors 8, 17, 21, 28, 31, 43, and 44 days old, respectively, and from 4 mm. to 2½ cm. in diameter. The older tumors were still growing when treated. In all, 55 tumors were brushed and 38 were held as controls. Tumors of 20 days and older, darkened and died in from 2 to 7 days after brushing. Young 8-day-old galls did not shrink or darken under the treatment, but were more or less inhibited and grew to only half the size of the controls.

Forty-nine-day-old tumors on *Bryophyllum pinnatum* (Lam.) Kurz, white, sound, and growing well, showed no effect from the full-strength a-methylnaphthalene, while 23-day-old tumors on the closely related plant *Kalanchoe daigremontiana* (Ham. and Per.) Berger darkened in 1 day and were dead in 2 weeks. The vessels, however, through these dead tumors and the dead stem tissue occurring just above and below them, continued to function for some time, as shown by the fact that the other parts of the plants lived more than a month.

A-methylnaphthalene, full strength, was brushed once on 27 daisy tumors 3 mm. to 1¼ cm. in diameter, aged 7, 27, 30, 36, and 49 days, respectively. All were killed in 2 to 7 days, except those 7 days old. These darkened and ceased growing but did not die. Thirty-one tumors were kept unbrushed for controls.

Two brushings of a 50 per cent emulsion of a-methylnaphthalene acted much the same as one brushing of the full-strength chemical by killing the older tumors and inhibiting the younger ones. Seventy marigold tumors 9, 15, 27, 28, 33, 41, and 44 days old and 18 daisy tumors 9, 27, 38, and 49 days old were treated, while 50 and 24 controls, respectively, were held for comparison.

When 2 per cent and 10 per cent acenaphthene, respectively, were added to 50 per cent emulsions of a-methylnaphthalene and brushed on bacterial plant tumors, the results were essentially those of a-methylnaphthalene emulsion alone. Growth in young marigold and daisy tumors usually was retarded after brushing with emulsions containing either the 2 or the 10 per cent acenaphthene and the tumors either did not grow at all or were smaller than the controls. There were some cases where small tumors, 4 to 8 mm. in diameter, after retardation, finally reached the size of the controls, and occasional tumors of this size were not retarded at all.

Tumors 30 days old and older, 1 cm. and over in diameter, usually darkened immediately and died in 2 to 7 days.

Experiments with Apiole

Apiole is an oily, non-volatile compound extracted from parsley seed. It is one of the few plant extracts said to produce polyploidy (7). Its effect on bacterial plant tumors was unlike that of colchicine.

Twelve Paris daisies, inoculated 17 days, bearing tumors 4–8 mm. in diameter, were brushed with full-strength apiole. Twelve marigold tumors, 13 days old, and 5–7 mm. in diameter, also were brushed. No change took place in the galls in 24 hours, so they were brushed again. In a month the treated tumors on both daisy and marigold were alive and had grown, but were smaller than the controls. Dwarfing followed the treatment but neither darkening nor death.

Fifteen marigold seedlings were watered with a 2 per cent apiole emulsion on 5 successive days, after which the stems were inoculated with *Bacterium tumefaciens.* The treated seedlings appeared less thrifty than the nontreated ones. That this difference in degree of vigor influenced the size of the galls is shown by the observation that a month after inoculation, the tumors on the apiole-treated plants were $\frac{1}{4}$ the size of those on the controls. About 3 weeks later the apiole-treated plants had sickened and died, but there was no prevention of tumor formation.

CHEMICALS NOT KNOWN TO CAUSE DOUBLING OF CHROMOSOME NUMBER

Two chemicals, normal heptyl aldehyde and methyl salicylate, not known to cause chromosome-doubling and used by animal pathologists in the control of mouse tumors, also were used in these experiments. With mouse tumors, growth is reported as slowed down; liquefaction, regression, and, even disappearance, of tumors following without affecting normal cells (16, 17).

Experiments with Methyl Salicylate

The tests consisted of (a) watering seedling marigold plants with emulsions of methyl salicylate to learn what effect this might have on tumor formation; (b) watering older marigold and daisy plants, having sizeable stem tumors, with emulsions of methyl salicylate; and (c) brushing tumors of different ages with methyl salicylate.

Marigold seedlings were watered for 5 successive days with different strengths of emulsions of methyl salicylate, after which the seedlings were inoculated with *Bacterium tumefaciens* to learn whether or not the formation of tumors would be prevented or perhaps delayed. The watering was continued for 12 days, each seedling receiving daily 20 to 25 cc. Of the 6 different strengths of emulsion tried, 0.3 per cent was the only one that did not kill the seedlings. One per cent, and higher, emulsions killed the seedlings in 1 to 2 days. In less than 2 weeks after inoculation tumors began to form on seedlings watered with 0.3 per cent emulsion. This was 5 to 6 days later than the controls, but the tumors soon reached the size of the controls.

Six large marigold plants, growing in 6-in. pots, with tumors 18 days old and 8 mm. to 1 cm. in diameter, were watered 18 times with 0.3 per cent emulsion of methyl salicylate. Twelve days after the watering began the tumors had become somewhat shrunken and slightly changed in color. No further change took place, even up to 22 days.

Six large Paris daisy plants with galls 21 days old, 5 mm. to 1 cm. in diameter, growing in 6-in. pots were watered with the 0.3 per cent emulsion at the same time as the marigold plants. Fifteen days after treatments

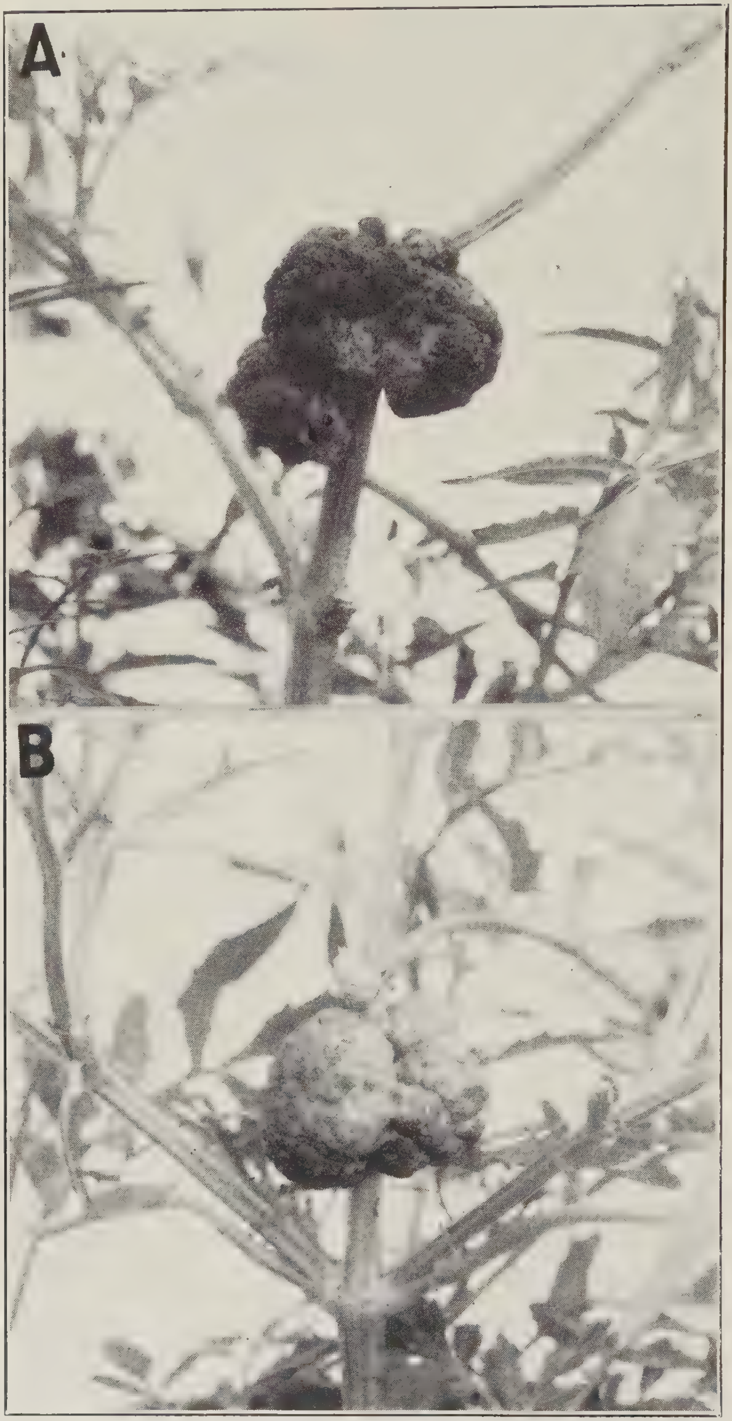

FIG. 2. A. *Bacterium tumefaciens* tumor on French marigold, 3 cm. in diameter, 44 days old and still growing when brushed, April 24, 1940, with methyl salicylate. Gall had blackened and shrunk some in one day when photographed, April 25, 1940; dead in 7 days. B. Control tumor on French marigold same age as A; inoculated with *Bact. tumefaciens,* March 11, 1940; photographed April 25, 1940. Both × 1.

started the galls began to shrink and darken; in 18 days they had become dry and dark; and in 24 they were dying. In 31 days the plants, themselves, began to show effects of the treatment by a yellowing of the leaves. At this time the control tumors were still growing.

Brushing young marigold tumors, 12 to 19 days old once with full strength of methyl salicylate, did not kill them. It retarded their growth for a time, but later they recovered and reached their full development. When marigold tumors were 30 to 44 days old, but still growing, they became shrunken, turned black, and died in less than a week (Fig. 2, A; control 2, B). French marigold tumors succumbed more readily than did those of the Guinea-gold marigold. Usually, if the tumors were larger than 1 cm. across, they died. The stems of treated plants were uninjured.

Marigold tumors 11 days old, 5 to 9 mm. in diameter, died subsequent to brushing 3 times at intervals of 1 day between brushings. This was a very severe treatment. The plants in this series brushed once remained alive and continued to grow.

Daisy tumors were far more sensitive to the full-strength methyl salicylate than were the marigold tumors. Some of them shrank nearly to half size, while the plant remained healthy. Daisy tumors 16 days old, 8 mm. to 1¼ cm. in diameter, and daisy galls 26 days old, 1½ to 2½ cm. in diameter, and still growing, were all dead 10 days after brushing. Very young daisy tumors 7 days old, 3–4 mm. in diameter, were inhibited in growth and became slightly darkened but did not die.

Full-strength methyl salicylate brushed on *Kalanchoe diagremontiana* tumors 2 months old, killed them but did not kill *Bryophyllum pinnatum* tumors of the same age. The Kalanchoe controls were alive and growing 4 to 5 months later, while the brushed ones were dead in less than 3 weeks. The treated Bryophyllum tumors were alive and growing after 5 months.

Experiments with N-heptyl Aldehyde

Twenty-five marigold seedlings were watered with n-heptyl aldehyde, each of 5 plants receiving 1,000, 700, 500, 200, and 100 parts per million, respectively. At the seventh watering the plants were inoculated with *Bacterium tumefaciens.* Thirteen days after commencing the experiment the treated seedlings were larger and more vigorous than the 25 nontreated controls. Nine days after inoculation, the galls on the treated seedlings were as large as those on the controls, and continued to grow equally well.

Other marigold seedlings were watered with 2 per cent, 1, ½, ¼, and ⅛ per cent n-heptyl aldehyde. These seedlings were able to withstand the ¼ and ⅛ per cent emulsions, and a few were not killed by the ½ per cent emulsion. Most of those watered with the 2, and 1 per cent emulsions died. After watering with the ¼ and ⅛ per cent emulsions, respectively, for 5 successive days, the plants were inoculated with *Bacterium tumefaciens* and the watering continued 5 more days. Tumors formed on the seedlings and grew as well as on the controls.

Brushing 25-day-old marigold tumors 1 to $1\frac{1}{2}$ cm. in diameter on 2 successive days with a 5 per cent emulsion of n-heptyl aldehyde was without effect. When 18-day-old marigold tumors, 5–7 mm. in diameter, were brushed with a 10 per cent emulsion there was some darkening from which the tumors recovered. Other marigold galls, 20 days old, brushed with the 10 per cent emulsion, became darkened for a time but recovered later. However, 1 brushing of a 20 per cent emulsion killed 12 marigold tumors 25 days old in 3 days (Fig. 1, E; control 1, F). Eight other marigold tumors 25 days old, treated with the 20 per cent emulsion mixed with bentonite, also were dead after 3 days. In addition, seven 35-day-old marigold tumors blackened and died in 3 days after 1 brushing with this mixture. There was a slight stem injury just above and below the tumors with the 20 per cent emulsion. Young marigold tumors 8 days old and 4–5 mm. in diameter were not affected by 1 brushing of the 20 per cent emulsion. No change occurred in the 12 galls in a week, and, in 10 days, they were nearly the size of the controls.

Full-strength n-heptyl aldehyde produced a rapid toxic effect on 12 marigold tumors 14 days old. Four hours after brushing, the tumors were shrunken and the stems darkened on either side of the outgrowth. In 24 hours the stems of 8 of the 12 plants had collapsed and bent over at the site brushed, and the tumors were dead.

Paris daisy tumors, 18 days old, brushed with full-strength n-heptyl aldehyde, were not affected in 24 hours as were the tumors on marigold plants, nor did the daisy stems bend over as did the marigold stems. In that time the daisy tumors had shrunk a little and a few leaves had dropped. In 2 days all 12 of the daisy tumors were shrunken and beginning to dry. In 11 days the tumors were alive and had grown some, but in 18 days 6 out of the 12 were dead. The other 6 continued slowly to increase in size.

Daisy tumors, 46 days old, varying in size from 8 mm. to $1\frac{1}{2}$ cm., were brushed with a 10 per cent n-heptyl aldehyde emulsion. In 3 days 6 of the 10 tumors brushed had shrunk and darkened and the leaf petioles near the galls also had darkened. In 10 days all the galls were blackened but still alive. In a month, however, they were dead, while the plants were still in very good condition.

Methyl salicylate and n-heptyl aldehyde seemed to have a toxic effect on both marigold and daisy tumors, according to the age and growing condition of the tumors and the strength of the emulsion used. In most cases young tumors were less susceptible to the chemicals than the older ones.

DISCUSSION

The results with acenaphthene in experiments with plant tumors were unlike those with colchicine. With the latter, there was stimulation of cell growth followed by cell collapse and death. Marigold tumors were practically unaffected by acenaphthene dissolved in dioxan, regardless of age or growing condition, while the daisy tumors, if not too young, became shrunken and blackened. Young daisy galls were unaffected. Out of a total of 164 marigold tumors brushed with acenaphthene dissolved in dioxan not one died. Any slight inhibition of growth that took place was overcome in a short time and the treated tumors soon reached the size of the controls.

Of the 44 daisy tumors brushed with acenaphthene dissolved in dioxan, 24 died. The other 20 lived and in most cases eventually reached the size of the controls. In no case did a young daisy tumor brushed with this solution show more than a slight darkening; indeed, those brushed when 31 days old, although at first somewhat shrunken and darkened by the treatment, entirely recovered.

It seems that the slowly penetrating acenaphthene is taken care of in a young growing plant by dispersion throughout its system. This gives a chance for the tumor to recover and continue its growth. Dispersion does not take place in the case of older daisy tumors. There were some differences in the reaction of tumors to the other chromosome-doubling chemicals, but, in the main, the action of these chemicals closely resembled that of acenaphthene.

According to Dermen's findings, the nuclei of young plant tumors seem not to be very much affected by acenaphthene, either because of a lack of penetration or from other causes. It is possible that death of older tumors may occur because there is not enough active growth for a systemic absorption of the chemical by the plant and a lytic effect follows because of its concentration in the tumors.

SUMMARY

The chromosome-doubling chemicals acenaphthene, a-methylnaphthalene, a-nitronaphthalene, 3–5-dibromopyridine, and apiole did not act on bacterial plant tumors produced by *Bacterium tumefaciens* in the same way as colchicine. The plant-tumor cells seemed to have their own specific response to the given chemical according to the species on which the tumor grew and, with a few exceptions, according to the age of the tumor. No excessive doubling of chromosomes in the nuclei of young tumors treated with these chemicals took place as did in young tumor nuclei treated with colchicine.

There was no stimulation of tumor growth by acenaphthene, except in those plant tumors brushed with this chemical dissolved in chlorinated-naphthalene and temporarily in those seedling marigolds watered with a saturated water solution of acenaphthene.

Acenaphthene dissolved in dioxan did not produce the noticeable stimulation of tumor development so characteristic of colchicine before inhibition and death take place. Three per cent acenaphthene in dioxan did not kill marigold tumors, young or old; it killed Paris daisy tumors 36 or more days old but not young daisy tumors.

Any inhibition of growth of marigold tumors by the action of acenaphthene dissolved in dioxan was usually overcome, and the treated tumors on reaching maturity were the size of the controls at maturity.

TABLE 1.—*Summary of reaction of crown gall to application of various chemicals*

Chemical amount and solvent	Host bearing crown gall				Application		Results	
	Kind of plant	Size of tumor	Tumors treated	Age of tumor when treated	Manner of treatment	Controls	After	Reaction
			Number	*Days*	*No. times brushed*	*No.*	*Days*	
3% Acenaphthene in dioxan[a]	French marigold	1 to 1¼ cm.	21	21	3	7	33	In 2 to 4 days after brushing, the tumors had darkened and shrunk a little. In 10 days they had recovered and were like control tumors.
‘‘ ‘‘	‘‘ ‘‘	¾ to 1 cm.	19	15	3	7	35	‘‘ ‘‘
‘‘ ‘‘	‘‘ ‘‘	4 to 5 mm.	5	11	4	5	22	‘‘ ‘‘
‘‘ ‘‘	‘‘ ‘‘	3 to 5 mm.	7	11	4	4	47	‘‘ ‘‘
‘‘ ‘‘	‘‘ ‘‘	3 to 4 mm.	6	7	3	6	12	‘‘ ‘‘
‘‘ ‘‘	‘‘ ‘‘	4 to 5 mm.	7	48	2	7	12	‘‘ ‘‘
‘‘ ‘‘	Paris daisy	1 to 1½ cm.	21	36	1	7	12	16 of the 21 tumors dead.
‘‘ ‘‘	‘‘ ‘‘	7 to 9 mm.	7	31	1	7	21	2 tumors dead; others alive.
‘‘ ‘‘	‘‘ ‘‘	1 to 1½ cm.	7	41	1	7	13	All tumors dead.
‘‘ ‘‘	‘‘ ‘‘	4 to 5 mm.	6	18	2	6	17	Young tumors not killed: were nearly as large as the controls.
3% Acenaphthene in dioxan	Paris daisy	1¼ to 1½ cm.	3	48	1	2	10	One tumor dead; the others growing.
5% Acenaphthene in dioxan	French marigold	1 to 1¼ cm.	7	22	4	7	37	Darkened and shrunk the first few days, then recovered and were like the control tumors.
‘‘ ‘‘ ‘‘	‘‘ ‘‘	¾ to 1 cm.	28	16	4	14	26	‘‘ ‘‘
10% ‘‘ ‘‘	‘‘ ‘‘	1 to 1¼ cm.	7	22	4	7	28	‘‘ ‘‘
‘‘ ‘‘ ‘‘	‘‘ ‘‘	¾ to 1 cm.	14	16	3	14	26	‘‘ ‘‘
‘‘ ‘‘ ‘‘	‘‘ ‘‘	4 to 5 mm.	5	11	4	5	22	‘‘ ‘‘

[a] To test the effect of moist chamber conditions, 31 other French marigolds with tumors of similar sizes and ages were treated with 3–5–10 per cent acenaphthene in dioxan. Results were the same as on those tumors kept in the open greenhouse.

TABLE 1.—(*Continued*)

Chemical amount and solvent	Host bearing crown gall				Application		Results	
	Kind of plant	Size of tumor	Tumors treated	Age of tumor when treated	Manner of treatment	Controls	After	Reaction
			Number	*Days*	*No. times brushed*	*No.*	*Days*	
1% Acenaphthene in lanolin	French marigold	1½ to 2 cm.	12	28	1	12 in pure lanolin	34	None
" " "	" "	¾ to 1 cm.	6	18	1	6 in lanolin	34	None
3% " "	" "	¾ to 1 cm.	12	15	1	7 in lanolin	30	"
Acenaphthene in filtered saturated water solution	" "	¾ to 1 cm.	55	16	2	7	20	"
" "	French marigold seedlings, 2½ inches tall	———	—	—	25 received chemical in soil 10 times, then inoculated with *Bacterium tumefaciens*	10	19	Tumors showed stimulation over controls for a week after forming; later they were same size as the controls.
5% Acenaphthene in chlorinated naphthalene	" "	¾ to 1½ cm.	28	22	2	10	20	Stimulation of tumor growth in those under 27 days old when treated.
" "	Paris daisy	8 mm. to 1 cm.	21	25	2	10	39	" "

TABLE 1.—(*Continued*)

Chemical amount and solvent	Host bearing crown gall				Application		Results	
	Kind of plant	Size of tumor	Tumors treated	Age of tumor when treated	Manner of treatment	Controls	After	Reaction
			Number	*Days*	*No. times brushed*	*No.*	*Days*	
1% 3–5 dibromopyridine in lanolin	French marigold	6 to 9 mm.	12	18	1	12 with pure lanolin	34	None
3–5 dibromopyridine in filtered saturated water solution	" "	1½ to 2 cm.	12	27	2	6	34	"
" "	Paris daisy	3 to 8 mm.	16	19	2	6	36	"
" "	" "	½ to 1 cm.	6	23	2	6	22	"
" "	Guinea gold marigold	1 to 1½ cm.	6	19	2	6	22	"
1% a-nitronaphthalene in lanolin	French marigold	1½ to 2 cm.	12	28	1	12 with pure lanolin	32	"
a-nitronaphthalene in saturated water solution	" "	6 to 9 mm.	12	18	2	6	34	"
" "	Paris daisy	3 to 8 mm.	6	19	2	6	36	"
" "	" "	1½ to 1 cm.	5	23	2	5	22	"
" "	Guinea gold marigold	1 to 1½ cm.	6	19	2	6	22	"
1% a-Methyl naphthalene in lanolin	French marigold	1½ to 2 cm.	12	28	2	12 with lanolin	34	"
a-Methyl naphthalene saturated water solution	French marigold	1 to 1¼ cm.	12	19	2	6	32	None
" "	Paris daisy	3 to 8 mm.	16	19	2	6	36	"

TABLE 1.—(*Continued*)

Chemical amount and solvent	Host bearing crown gall				Application		Results	
	Kind of plant	Size of tumor	Tumors treated	Age of tumor when treated	Manner of treatment	Controls	After	Reaction
			Number	*Days*	*No. times brushed*	*No.*	*Days*	
a-Methylnaphthalene 50% emulsion	French marigold	1¾ to 2½ cm.	3	44	1	6	14	Tumors dark and shrunk in 2 days; dead.
" "	" "	1½ to 2½ cm.	7	41	2	7	21	Dark in 1 day; dead.
" "	" "	¾ to 1¼ cm.	21	33	7 brushed 2 times	7	24	Dark in one day; 3 dead; those brushed twice were half the size of those brushed once.
" "	" "	¾ to 1¼ cm.	3	28	1	6	27	Tumors dark in one day; half of them dead later, others barely alive.
" "	" "	¾ to 1 cm.	12	15	6 brushed 2 times	12	30	Growth inhibited at once in those brushed twice; were ¾ size of control tumor in a month. None dead.
" "	" "	4 to 5 mm.	12	9	1	6	27	Tumors nearly size of controls.
" "	Paris daisy	1¼ to 2 cm.	3	49	1	6	2	Tumors dark in one day; dead in 2 days.
" "	" "	1 to 1½ cm.	6	38	1	6	5	Tumors dark and shrunk in one day; dead.
" "	" "	6 to 10 mm.	3	27	1	6	7	Tumors dark in one day; dead.
" "	" "	4 to 5 mm.	6	9	1	6	41	Tumors slightly dark in one day; were inhibited, but alive.
a-Methylnaphthalene full strength	French marigold	¾ to 2½ cm.	3	44	1	6	7	Dead.
" "	" "	1½ to 2 cm.	5	43	1	5	5	Dead.
" "	" "	1 to 2 cm.	12	31	1	6	4	Dead.

TABLE 1.—(*Continued*)

Chemical amount and solvent	Host bearing crown gall				Application		Results	
	Kind of plant	Size of tumor	Tumors treated	Age of tumor when treated	Manner of treatment	Controls	After	Reaction
			Number	*Days*	*No. times brushed*	*No.*	*Days*	
a-Methylnaphthalene full strength	French marigold	$1\frac{1}{4}$ to $1\frac{1}{2}$ cm.	3	28	1	6	7	Dead.
" "	" "	$\frac{3}{4}$ to $1\frac{1}{4}$ cm.	7	21	1	5	4	Dead.
" "	" "	4 to 8 mm.	18	17	1	5	28	Tumors browned in 2 days but later recovered and grew to size of controls.
" "	" "	4 to 5 mm.	7	8	1	5	30	Tumors were dark and smaller than control tumors.
" "	*Bryophyllum pinnatum*	5 mm. to 1 cm.	6	49	1	4	30	No effect on tumors.
" "	*Kalanchoe daigremontiana*	$\frac{3}{4}$ to 1 cm.	6	23	1	4	14	Tumors darkened in one day; dead in 2 weeks.
" "	Paris daisy	$1\frac{1}{2}$ to 2 cm.	3	49	1	6	2	Tumors dead.
" "	" "	1 to $1\frac{1}{4}$ cm.	6	36	1	7	7	" "
" "	" "	$\frac{3}{4}$ to $1\frac{1}{4}$ cm.	9	30	1	5	4	" "
" "	" "	6 to 10 mm.	3	27	1	6	2	" "
" "	" "	3 to 4 mm.	6	7	1	7	21	Tumors inhibited.
Apiole 2% emulsion	French marigold seedlings	Inoculated after treating	—	—	Emulsion on seedlings for 5 days	10	35	Treated seedlings were inoculated with *Bact. tumefaciens* after 5 treatments. Tumors formed; $\frac{1}{4}$ size of control tumors.
Apiole, full strength	Guinea gold marigold	5 to 7 mm.	12	13	Brushed 2 times	6	42	Slowing down of tumor formation and dwarfing.
" "	Paris daisy	4 to 8 mm.	12	17	2	6	46	" "

TABLE 1.—(*Continued*)

Chemical amount and solvent	Host bearing crown gall				Application			Results
	Kind of plant	Size of tumor	Tumors treated	Age of tumor when treated	Manner of treatment	Controls	After	Reaction
			Number	*Days*	*No. times brushed*	*No.*	*Days*	
Methyl salicylate 0.3% emulsion	French marigold 10 seedlings	Inoculation after treating	—	—	Watered seedlings with emulsion 12 days	20	21	Tumors formed 5 to 6 days later on treated seedlings than on controls. Reached control-tumor size later.
" "	French marigold plants	8 mm. to 1 cm.	6	18	Watered with emulsion 18 times	3	22	Tumors had darkened and shrunken some in 12 days. No further change.
" "	Paris daisy plants	½ to 1 cm.	6	21	" "	3	24	Tumors dying in 24 days.
Methyl salicylate full strength	French marigold	1 to 1¾ cm.	12	24	Brushed 2 times	6	24	Tumors dead.
" "	" "	½ to 1¼ cm.	8	19	1	6	48	Tumors alive.
" "	" "	3 to 3½ cm.	3	44	1	3	6	Tumors dead.
" "	" "	2 to 2½ cm.	3	30	1	3	6	" "
" "	" "	1 to 1¼ cm.	6	25	1	4	22	" "
" "	Guinea gold marigold	8 to 10 mm.	6	14	2	6	35	Three tumors dead, others inhibited.
" "	" "	5 to 7 mm.	18	15	3	7	17	Tumors dead.
" "	" "	5 to 8 mm.	9	19	1	7	18	Tumors alive and growing.
" "	" "	5 to 9 mm.	12	11	1	7	17	Tumors alive; darkened.
" "	" "	5 to 9 mm.	6	11	3	7	17	" dead.
" "	" "	2 to 2½ cm.	6	57	1	3	2	" "
" "	" "	6 mm. to 1 cm.	24	23	1	6	16	" darkened but growing.
" "	" "	6 mm. to 1 cm.	6	23	2	6	8	" dying.
" "	" "	6 mm. to 1 cm.	6	23	3	6	8	" dead.
" "	" "	6 mm. to 1 cm.	6	25	4	6	8	" "
" "	Paris daisy	3 to 4 mm.	5	7	1	3	33	" darkened and inhibited.

TABLE 1.—(*Continued*)

Chemical amount and solvent	Host bearing crown gall				Application		Results	
	Kind of plant	Size of tumor	Tumors treated	Age of tumor when treated	Manner of treatment	Controls	After	Reaction
			Number	*Days*	*No. times brushed*	*No.*	*Days*	
Methyl salicylate full strength	Paris daisy	6 to 8 mm.	6	18	1	6	19	5 tumors dead, one alive.
" "	" "	8 mm. to $1\frac{1}{4}$ cm.	3	16	1	3	10	Tumors dead.
" "	" "	1 to $1\frac{1}{4}$ cm.	3	36	1	3	2	" "
" "	" "	$1\frac{1}{2}$ to $2\frac{1}{2}$ cm.	6	26	1	6	10	" "
" "	*Kalanchoe daigremontiana*	$1\frac{1}{2}$ to 2 cm.	3	58	1	3	20	" "
" "	*Bryophyllum pinnatum*	$\frac{1}{2}$ to 1 cm.	3	60	1	3	150	" alive and growing.
n-heptyl aldehyde 1000 parts per million	French marigold seedlings	Inoculated after treating	—	—	7 seedlings treated 7 times, then inoculated	25	18	Treated seedlings larger than control seedlings. No checking of tumor growth.
n-heptyl aldehyde 700 parts per million	French marigold	Inoculated after treating	—	—	7 seedlings treated 7 times, then inoculated	25	18	Treated seedlings larger than control seedlings. No checking of tumor growth.
n-heptyl aldehyde 500 parts per million	" "	" "	—	—	" "	25	18	" "
n-heptyl aldehyde 200 parts per million	" "	" "	—	—	" "	25	18	" "
n-heptyl aldehyde $\frac{1}{4}$% emulsion	" "	" "	—	—	Treated 10 times. Inoc. after 5 treatments	5	22	" "
n-heptyl aldehyde $\frac{1}{8}$% emulsion	" "	" "	—	—	" "	5	22	" "

TABLE 1.—(*Continued*)

Chemical amount and solvent	Host bearing crown gall				Application			Results
	Kind of plant	Size of tumor	Tumors treated	Age of tumor when treated	Manner of treatment	Controls	After	Reaction
			No.	*Days*	*No. times brushed*	*No.*	*Days*	
n-heptyl aldehyde 5% emulsion	French marigold large plants	Tumors 1 to $1\frac{1}{2}$ cm.	8	25	Brushed 2 times	4	12	No inhibition of tumor growth.
n-heptyl aldehyde 10% emulsion	Guinea gold marigold	5 to 7 mm.	18	15	2	12	24	" " " " "
" "	" "	5 to 9 mm.	12	20	3	12	22	" " " " "
" "	Paris daisy	8 mm. to $1\frac{1}{4}$ cm.	10	46	1	5	24	Tumors dead.
n-heptyl aldehyde 20% emulsion	Guinea gold marigold	1 to $1\frac{1}{4}$ cm.	12	25	1	6	20	" "
" "	" "	$1\frac{1}{2}$ to $2\frac{1}{4}$ cm.	7	35	1	6	13	" "
" "	French marigold	4 to 5 mm.	12	8	1	6	40	Tumors not inhibited or killed; too young to be affected.
n-heptyl aldehyde in bentonite	Guinea gold marigold	1 to $1\frac{1}{4}$ cm.	8	25	1	6	20	All tumors killed.
n-heptyl aldehyde full strength	" "	5 to 7 mm.	12	14	1	6	5	All tumors dead.
" "	Paris daisy	4 to 8 mm.	12	18	1	6	12	Tumors alive but $\frac{1}{3}$ size of control tumors.

Dwarfing occurred when apiole was brushed on tumors, but there was no darkening or death.

Full-strength a-methylnaphthalene brushed on young marigold and Paris daisy tumors inhibited further growth; it killed old but growing tumors on both hosts. A 50 per cent emulsion had much the same effect. The two related plants *Bryophyllum pinnatum* and *Kalanchoe daigremontiana* responded differently to a a-methylnaphthalene brushed on stem tumors. In a month there was no effect on Bryophyllum, while Kalanchoe tumors were dead in 2 weeks.

A-nitronaphthalene and 3–5-dibromopyridine were used only in saturated water solutions and in lanolin paste. Young and old Paris daisy and marigold tumors were brushed with these chemicals, with negative results. Two chemicals, heptyl aldehyde and methyl salicylate, not known to be polyploidizing agents, but used by animal pathologists in the control of mouse tumors, were also applied to bacterial plant tumors. Brushing normal heptyl aldehyde full strength on 12 marigold tumors, 14 days old, killed them in 24 hours, while 18-day-old daisy tumors became slightly shrunken. In 3 weeks only half of the 18-day-old tumors were dead; the other 6 tumors continued to live and grow. A 20 per cent emulsion of n-heptyl aldehyde killed 20 marigold tumors, 25 days old, in 3 days; 8-day-old marigold tumors were not affected. A 10 per cent emulsion of this chemical killed 10 46-day-old daisy tumors.

Full strength a-methyl salicylate killed 30- to 44-day-old marigold tumors, but not 12- to 15-day-old marigold tumors. Paris daisy tumors, 16 to 26 days old, were killed with a-methyl salicylate, and tumors 7 days old when brushed were inhibited.

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EYE–SPOT OF NAPIER GRASS IN HAWAII, CAUSED BY HELMINTHOSPORIUM SACCHARI[1]

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INTRODUCTION

Napier grass (*Pennisetum purpureum* Schum.) was first introduced into Hawaii in 1912 from Africa (31); since that time it has become highly esteemed by local ranchers as a soiling crop and for pastures. In October, 1939, specimens of diseased Napier, or elephant grass as it is also called, which showed lesions on the leaves, leaf sheaths, and stems, together with a general killing of the plant, were received from Kona, on the Island of Hawaii. Since the original outbreak, the disease has spread to all of the islands of the Hawaiian group, causing appreciable losses. In the present paper facts concerning the disease and its causal agent *Helminthosporium sacchari* (van Breda de Haan) Butler, are presented.

SUSCEPTS

Plants Affected

Napier grass and sugar cane (*Saccharum officinarum*) are the plants most commonly affected by this disease, but Mitra (20) has reported that the leaves of wheat, barley, oats, maize, sorghum, rice, and *Pennisetum typhoideum* are also susceptible. The writer has demonstrated that *P. setosum* is not susceptible, and Paterson (24) has reported that Guatemala grass (*Tripsacum laxum*), growing alongside severely diseased Napier, showed no symptoms of disease.

Varietal Susceptibility

Stokes and Ritchey (28) report that resistant and immune strains of Napier grass are known in Florida. Voorhees (29) inoculated a number of these strains and found that none of the so-called "immune" strains became infected, whereas all strains originating from susceptible Napier developed the disease. Merker grass, a variety of Napier (30) and several reciprocal Napier × Merker crosses developed by the Hawaii Agricultural Experiment Station are resistant to the disease (22).

THE DISEASE

Name

The name "eye-spot" was assigned to this disease of Napier grass by Voorhees (29), who believed that it was caused by the fungus responsible for eye-spot of sugar cane. The writer concurs in this opinion, and the name eye-spot is retained and used throughout this paper to denote the disease of Napier as it occurs in Hawaii.

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History and Range

Paterson (24) probably is referring to eye-spot when he describes a disease of Napier in Trinidad in 1933, which produced elongated, reddish-brown spots on the leaves. Ultimately the leaves were killed and the plant was checked in its growth. Stell (26) also records eye-spot on Napier in Trinidad; adjacent sugar cane was unaffected. In 1935, Leukel and Camp (15) mention that yields of Napier in Florida were severely reduced by a disease, which Stokes and Ritchey (28) and Voorhees (29) later demonstrated was eye-spot. The disease probably occurs in other parts of the tropics on Napier, but no further references have been found in the literature.

In Hawaii eye-spot undoubtedly was present some months prior to its discovery, for the first specimens examined were in advanced stages of the disease. According to statements by ranchers of long standing in the Kona community, many years ago a similar outbreak occurred and fields of Napier were destroyed; later, the disease disappeared. Whether or not the disease in question was eye-spot is uncertain, but it is significant to note that Paterson (24) records that sudden fluctuations in the severity of eye-spot are not uncommon.

Importance

This disease may cause losses in any one or all of several ways. The leaves may be killed prematurely or be so badly affected that they dry out and become unpalatable. On the stems, cankers are formed which interfere with normal physiological activities of the grass and cause premature drying and shedding of foliage. Cankered stems quickly become pithy and hollow and unfit for stock feed. Young buds, at or near the soil level, may be weakened or killed outright. Secondary organisms enter the rootstock by way of these dying or dead buds, and initiate decay.

Losses due to this disease are undoubtedly of appreciable magnitude, but no monetary estimate has been made. Where the disease has appeared in severe form, entire plantings have been rendered useless for fodder, and many have been plowed and replanted with other grasses.

Symptomatology

Morphologic Symptoms on the Leaves. Initial spots appear as small, yellowed areas within normal green tissues; later, the spots turn reddish-brown and may exhibit a yellow halo. The center of the spots may be lighter brown than the periphery, and sometimes one or more rings are present to give a zoning effect. In shape, the spots are oval and fairly regular in outline except where two or more coalesce to produce an irregularly shaped lesion (Fig. 1, A). They measure 1 to 6 mm. long by 0.5 to 2 mm. wide, with the greater dimension always parallel to the long axis of the leaf, and are very similar to spots described by Voorhees (29).

Runners or streaks may be present on diseased leaves (Fig. 1, B), extending from the primary lesion for several inches toward the apex of the leaf. Their color is essentially the same as that of old lesions, and the tissues involved are obviously dead. Compared to the damage produced on sugar cane in Hawaii by eye-spot runners (16), the damage on Napier caused by runners is slight. Streaks of dead tissue not associated with primary lesions, accompanied by anthocyanescence, may also be produced on the leaves. These streaks usually are found on plants with cankered stems and probably are due to disturbance of the normal functioning of the vascular system of the stem by the pathogen.

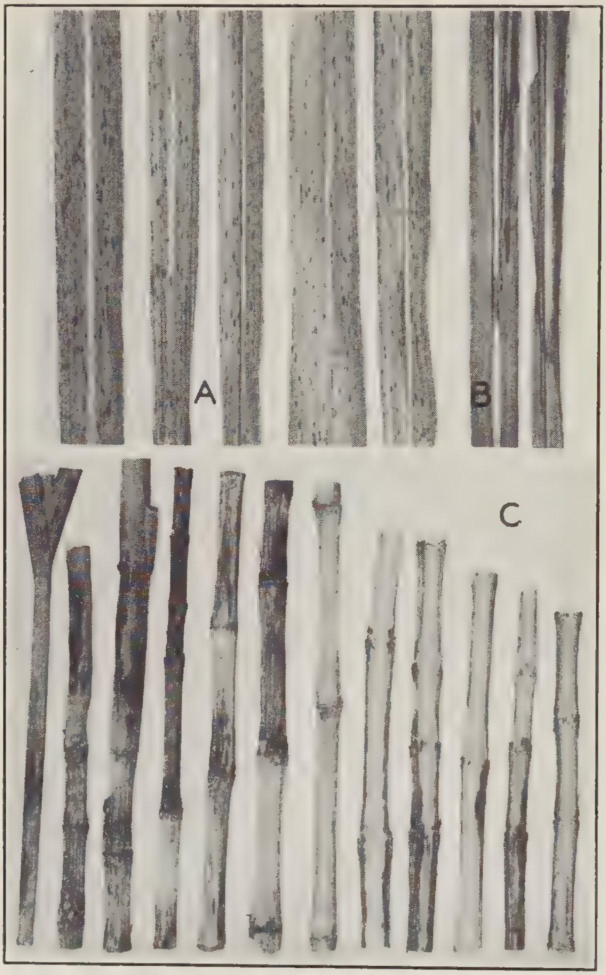

FIG. 1. Eye-spot caused by *Helminthosporium sacchari*, on Napier grass. A and B. On leaves; in B, runners are shown. C. On leaf sheaths and stems; note cankers, external and internal, and withering of stem. Healthy stem in center.

The effect of the disease is more pronounced on old leaves than on young, and the growing tip is seldom attacked. This is in opposition to findings by Martin (16) and others in Hawaii for the effect of eye-spot on susceptible varieties of sugar cane. Severe infection causes the leaves to wither, dry out, and die prematurely in acropetal succession: eventually, most of the leaves hang pendent or fall to the ground and the stem is bare except for a tuft of three or four leaves.

On the Leaf Sheaths. Lesions are commonly found on the leaf sheaths (Fig. 1, C). They are larger than spots on the leaves, lighter in color, and more diffuse in outline. Penetration of the sheath may take place and the stem itself be attacked.

On the Stems. Stem lesions are more elongate than leaf lesions and correspondingly narrower (Fig. 1, C). They are smooth at first, regular in outline and reddish-brown, with or without a lighter-colored center; later they become sunken, irregular in shape, and bluish-purple to black; the border may be grayish-white or retain the original reddish-brown color. Lesions are found from ground level to several feet above the soil, but usually occur on the first 6 inches of the stem. The nodes are more commonly attacked than the internodes. Affected stems are shrunken, pithy, and partly or completely hollow; when split longitudinally in the region of cankers, the interior of the stem is seen to be diseased or dead in part or in whole (Fig. 1, C).

On the Crown. The crown is seldom affected; occasionally, however, diseased tissue can be found and the basal buds are withered and darkened and their subsequent growth is stunted. The roots are not thought to be attacked, and Voorhees (29) mentions no symptoms on these parts. However, plants affected with eye-spot have weakened root systems and are quickly knocked over and trampled by grazing animals. *Marasmius* sp. (*sacchari?*) is present in decayed Napier roots; this fungus possibly attacks plants debilitated by eye-spot.

On the Plant as a Whole. From a distance, badly diseased Napier appears to be blasted by lightning or burned by fire. Plantings that have been diseased for some time show an abundance of partly developed adventitious side shoots, in various stages of destruction by the disease.

ETIOLOGY

Name, History, and Classification of the Pathogen

The organism causing eye-spot of Napier in Hawaii is believed to be the same as that producing eye-spot of Napier in Florida, which Stokes and Ritchey (28) and Voorhees (29) state is *Helminthosporium ocellum* Faris. This fungus was claimed by Faris (9), whose data were accepted by Bourne (2), to be the cause of eye-spot of sugar cane in Florida, Cuba, Santo Domingo, Puerto Rico, and Hawaii. For many years previously, sugar-cane pathologists had ascribed eye-spot to *H. sacchari* Butler (4), spoken of in India, from which it was originally described in 1913, as the cause of "helminthosporiose." Faris' arguments in favor of the erection of a new species were based on a difference in symptom expression and spore length. *H. sacchari* was reported to occur equally on the midrib and the thin part of the leaf and to have a mean spore length of 47.5 μ, while Faris found that

H. ocellum occurred more frequently on the thin part of the leaf than elsewhere, and the mean spore length was 69 μ. Investigators prior to Faris had similarly found that the spore length reported by Butler was unusually low and that *H. sacchari*, or *Cercospora sacchari* van Breda de Haan (3), as it was incorrectly named, produced spores that varied in mean length from 61 μ to 78 μ, depending on the observer (Table 1).

Mitra (20), working in India, has more recently shown that Butler's spore measurements do not indicate the full range of variability of *H. sacchari*, and that spore length, color, and curvature are determined by temperature and by the substrate on which the fungus is grown. Instead of 47.5 μ as the mean spore length, the figure should be advanced to 68.3 μ, which is almost identical with what Faris found. In a second paper Mitra (21) concludes that *H. sacchari* and its strains (saltants) have a range of variation in spore dimensions sufficiently wide to include all forms of *Helminthosporium* causing eye-spot.

McRae (19), also working in India, made a comparative study of the physiology of *H. sacchari*, *H. ocellum*, *H. stenospilum* Drechsler,[2] now known as *Cochliobolus stenospilus* (Carp.) M. and Y., and the unidentified species of *Helminthosporium* of Priode (25). The last 3 species were obtained from Florida, the *H. sacchari* from India. McRae's findings can be summarized as follows: (a) On certain media the species were much alike and appeared to be closely allied, while on other media they differed in certain cultural characteristics; (b) the range of spore measurements of the species and their strains under standard conditions fell within the range of *H. sacchari* and its saltants; (c) *H. sacchari* and *H. ocellum* were much alike, and from their growth in culture, spore length, and septation appeared to be allied; and (d) the type and color of lesions produced on cane leaves by the species and their saltants differed so slightly from those of each other that it was almost impossible to distinguish the fungi by their spot characters. McRae considered *H. ocellum* to be identical with *H. sacchari* but a different strain and recommended that the name *H. sacchari* Butler, with its amplified description by Mitra (20), be maintained for

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[2] McRae (19) found one mutant of *Helminthosporium sacchari*, which produced spores similar in size and septation to spores of *H. stenospilum;* Drechsler (8) separated the two on the basis of the larger spores of *H. stenospilum* and a difference in symptoms with *H. stenospilum* causing brown stripe. Martin (16) reports that measurements of the spores of the brown-stripe fungus in Hawaii demonstrated that it was difficult to distinguish brown stripe from eye-spot. The writer isolated *H. stenospilum* from authentic cane material supplied by J. P. Martin, and measured the spores produced in culture. The following results were obtained:

At 21° C., on corn-meal agar: 25.6–73.6 μ (weighted mean 46.1 μ) by 9.6–18.4 μ (weighted mean 13.0 μ) with 3–9 septa per spore (weighted mean 5.4).

Ditto, on 1-per cent-sucrose agar: 32.0–76.8 μ (weighted mean 51.3 μ) by 12.0–20.0 μ (weighted mean 14.9 μ) with 3–8 septa per spore (weighted mean 5.8).

Ditto, nutrient agar: no spores produced after one month.

At 28° C., no spores were produced on any one of above agars, after one month.

In color and shape, the spores of *H. stenospilum* closely resembled those of *H. sacchari* and their sizes were not unlike. This corroborates Martin's (16) previous rather unorthodox findings.

TABLE 1.—*Length, width, septation, color, and shape of spores of H. sacchari as found by various investigators*

Investigator	Name assigned to pathogen	Length in μ			Width in μ			Septa			Color and shape
		Range	Mean	Weighted mean	Range	Mean	Weighted mean	Range	Mean	Weighted mean	
Kruger	*C. sacchari*	32.4– 90	......	78.5	9.0–14.4	12.5		5– 8	6.5		Smoky-brown
Breda de Haan	*C. sacchari*	60.0– 80	70.0	......	9.0–12.0	10.5		5– 8	6.5		Reddish brown, lengthened oval
Cobb	*C. sacchari*	50.0– 72[a]	61.0[a]	......	11.0–14.0[a]	12.5[a]	......	3– 9[a]	......	7.0	Dark, curved
Larsen	*C. sacchari*	53.0– 82	67.5		9.0–15.0	12.0	......	4–10	8.0		Dark, curved
Butler	*H. sacchari*	35.0– 60	47.5	......	8.5–12.0	10.2	......	3–10	6.5		Olive-green to brown, cylindrical or long elliptical
Cook (1924)	*H. sacchari*	45.0–110	77.5	......	......	12.0	......	5–11	8.0	......	......
Cook (1924)	*H. sacchari*	30.0– 95	62.5	......	12.0–15.0	13.5	......	4–10	7.0	......	......
Cook (1927)	*H. sacchari*	22.0– 92	......	58.7	6.6–15.0	......	11.1	3–11	7.0	......	......
Johnson and Stevenson	*H. sacchari*	32.0– 90	61.0	......	9.0–14.0	11.5	......	several	......	......	......
Bourne (1922)	*H. sacchari*	Up to 75	......	......	......	......	......	......	......	......	......
Bourne (1934)	*H. ocellum*	24.0– 92	......	58.7	11.1–16.7		14.0	3– 9		6.7	Light smoky yellow-brown, slightly curved
Faris (1928)	*H. ocellum*	32.0–110	......	69.0	6.6–20.8		12.7	3–10	6.5		Light smoky yellow-brown, slightly curved
Faris (1928)	*H. ocellum*	29.0– 94	......	69.0	9.0–21.0		12.7	3–10		6.7	Light smoky yellow-brown, slightly curved
Faris (1928)	*H. sacchari*	19.0– 71	......	52.6	9.4–15.0	......	12.5	......	5.5	......	......
Drechsler	*H. ocellum*	32.0–103	......	71.0	9.0–17.0		14.0	3–10		6.7	Fuliginous
Voorhees	*H. ocellum*	28.0– 90	......	56.4	8.0–14.0		11.1	3– 9		6.5	Light smoky yellow-brown, slightly curved
Matsumoto and Yamamoto	*H. sacchari*	33.0–101	62–75	......	11.0–16.0	13–15	......	3–10	6.8		Smoky-gray to grayish-olive or citrine-drab, long elliptical or fusiform
Martin	*H. sacchari*	......	......	......	......	......	......	4– 8	......	......	Slightly curved
Mitra (1931)	*H. sacchari*	30.0–112	......	68.3	11.5–18.5	......	15.3	3–11		6.9	Yellowish, light olivaceous- to dark smoky-brown with often a tinge of greenness; cylindrical, long elliptical, often slightly curved
Stevenson and Rands	*H. sacchari*	22.0–110	......	......	9.0–21.0	......	......	3–10	......	......	Olive-green to brown, cylindrical, oblong or elliptical, often slightly curved

[a] Obtained by inspection and measurement of drawings by Cobb (5).

eye-spot in Cuba, Florida, etc. Stevenson and Rands (27) accept the conclusions of Mitra (20, 21), and McRae (19) and list *H. ocellum* as a synonym of *H. sacchari*, which is stated to be a highly variable fungus and apparently existing as a number of different strains with saltation common.

The conidiophores of *H. sacchari* from Napier are nonbranched, many septate, with angular inequalities or geniculations marking the insertion of an originally apical spore. The spores, to be described in more detail later, are straight, moderately or markedly curved, long-elliptical, with the greatest diameter near the middle or at one end, gradually tapering to rounded or slightly pointed ends, and many septate: they germinate from one end or from both ends. In color they vary from light to dark brown.

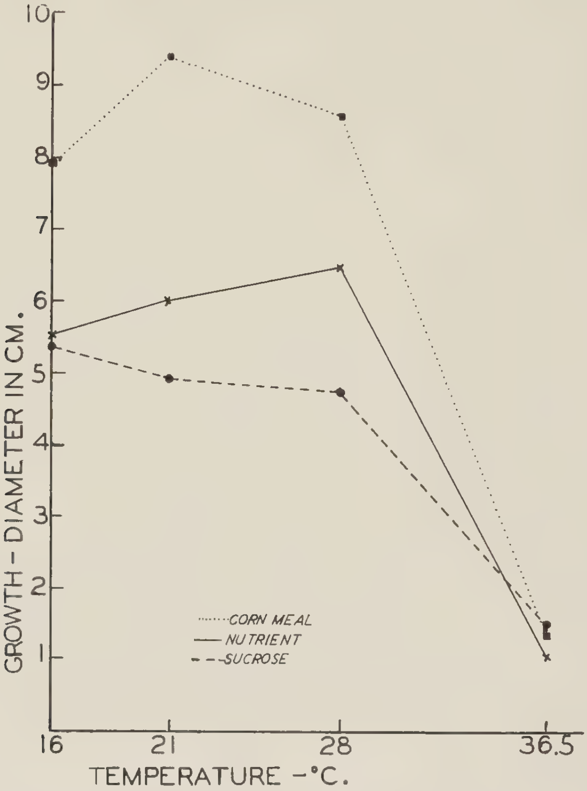

FIG. 2. Effect of temperature on growth of *Helminthosporium sacchari* when cultured on corn-meal agar, standard-nutrient agar and sucrose agar.

Physiology

When the disease appeared on Napier in Hawaii, diseased material was obtained from all of the principal islands of the Hawaiian group, and in most cases from several localities on each island, and the *Helminthosporium* cultured. Ten isolates were thus obtained. A single isolate of *H. sacchari* was cultured from cane diseased with eye-spot, obtained from the grounds of the Hawaiian Sugar Planters' Association Experiment Station, Honolulu; later, the writer was given 2 additional cultures of *H. sacchari,* isolated from the same locality several years previously, by J. P. Martin, plant pathologist of the sugar station. Napier isolates are hereafter designated in the text as *H. sacchari* (N) and the cane isolate is designated *H. sacchari* (C). The isolates of *H. sacchari* (N) and of *H. sacchari* (C) were studied side by side on various culture media,[3] at different temperatures, to determine growth characters and the formation, size, and color of spores, in alternating light and darkness and in continuous darkness. All media, except Shear's corn-meal agar, were adjusted to pH 7.0.

Effect of Temperature

Growth. The effect of temperature on the growth of *Helminthosporium sacchari* (N) (Fig. 2) was determined, using a single isolate obtained from Kawailoa, Oahu. The fungus was transferred on blocks of corn-meal agar, as nearly equal in size as possible to duplicate plates of standard nutrient, corn-meal, and 1 per cent-sucrose agars, which were placed at 36.5°, 28°, 21° and 16° C. in continuous darkness for 8 days, when the growths obtained were measured. Results, illustrated in figure 2, indicate that *H. sacchari* (N) grows well over the range 16 to 28° C. Somewhat less growth is obtained at the lower than at the higher temperature, except on sucrose where temperature affects the type, as well as the amount, of growth. This point is discussed later. At 36.5° C., growth is slow. The optimum temperature seems to lie between 21° and 28° C. No comparable studies were made with *H. sacchari* (C), but good growth was obtained with all 3 cultures (single isolate) on all media at 21–28° C. Growth was more rapid at the higher figure. Halma and Fawcett (10) have previously shown that *H. sacchari* (C), from Hawaii, has an optimum between 20° and 29° C., with more rapid growth at 29° than at 20° C. Bourne (2) found that 23.5° was optimum for *H. ocellum* from cane, but likewise obtained good growth from 21° to 28° C., while Mitra (20) found that the optimum for *H. sacchari,* in India, was 30° C.

On standard nutrient and on sucrose agars, temperature not only determined the amount of growth of *Helminthosporium sacchari* (N) but also the type of growth. On the former medium, white aerial mycelium was produced at 21° and 28° C., but not at 16° or 36.5° C., and was less abundant at 21° than at 28° C. On sucrose, at 21° and 28° C., the fungus exhibited sectorial growth somewhat similar to that described by Bourne (2) at 28° to 31° C. for *H. ocellum.* At 16° C. growth was somewhat similar to Bourne's picture of the fungus at 23° C.

[3] Potato-dextrose agar— 200 g. potatoes, 10 g. dextrose, 18 g. agar, 1000 cc. water.
Standard nutrient agar—3 g. beef extract, 5 g. peptone, 18 g. agar, 1000 cc. water.
Corn-meal agar— 20 g. corn meal, 18 g. agar, 1000 cc. water (Shear's formula).
Nutrient-dextrose agar—3 g. beef extract, 5 g. peptone, 20 g. dextrose, 18 g. agar, 1000 cc. water.
1 per cent sucrose agar—10 g. sucrose, 18 g. agar, 1000 cc. water.

Spore Formation, Size, and Color

Bourne (2) has reported that *Helminthosporium ocellum* fails to form spores at temperatures up to and including 10.6° C., and at 35° and 38° C. All isolates from Napier and from sugar cane produced spores at 21° and at 28° C. The Kawailoa isolate of *H. sacchari,* which was also studied at 16° and at 36.5° C., produced spores less abundantly at 16° than at 21° or 28°, and did not sporulate at 36.5° C.

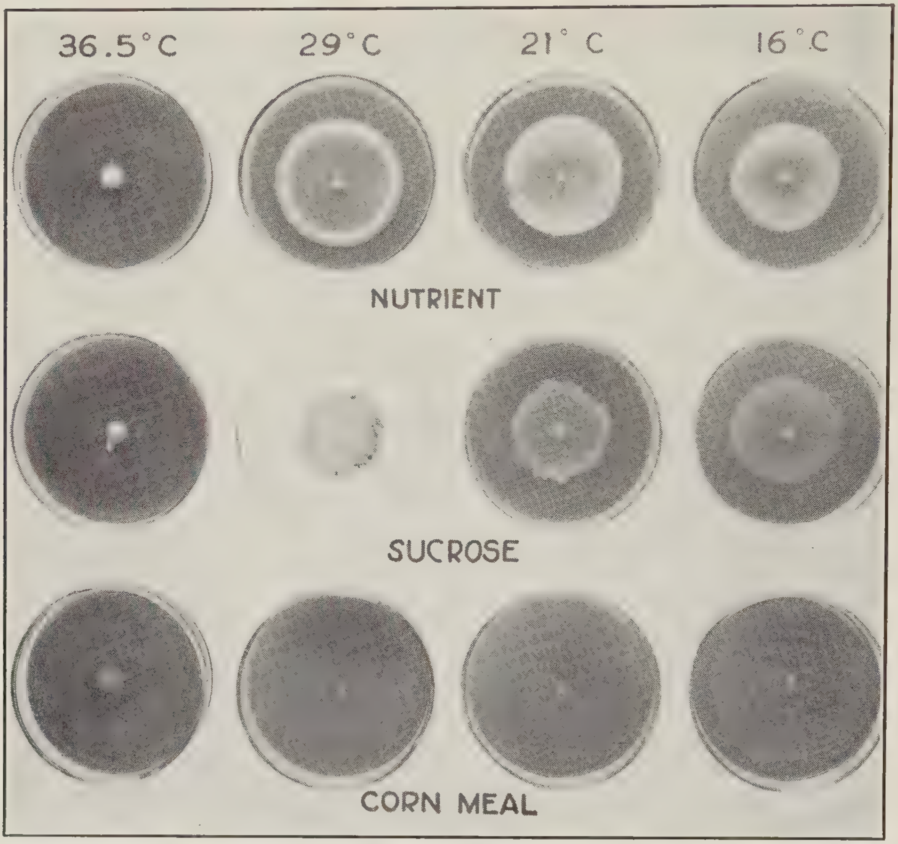

FIG. 3. Effect of temperature and substrate on growth of *Helminthosporium sacchari* (N), isolated from Kawailoa, Oahu; growth in continuous darkness for 8 days.

One hundred fifty spores of each isolate were measured, on each medium, at 21° and at 28° C. Results are summarized in table 2 as ranges and weighted means of length, width, and septation. The color of spores is also recorded. Between different isolates of *Helminthosporium sacchari* (N), considerable variation in spore size, under similar environmental conditions, was found; less variation was found within the single isolate (3 cultures) of *H. sacchari* (C).

The spores of *Helminthosporium sacchari* (N) are longer, narrower and possess more septa when formed at 28° than when produced at 21° C. Con-

TABLE 2.—*Length, width, septation, and color of spores of H. sacchari, from Napier grass and from sugar cane, on four different media at two different temperatures. One hundred fifty spores were measured from each isolate, on each medium, at each temperature*

Plant from which fungus isolated	Number of isolates studied	Medium	Temp. °C.	Length in μ		Width in μ		Number of septa		Color of spores
				Range	Weighted mean	Range	Weighted mean	Range	Weighted mean	
Napier grass	10	Sucrose	28	22.4–128.0	61.6	8.8–16.0	11.6	2–13	6.7	Yellow brown, smoky brown, or dark brown
	10	Corn meal	21	19.2– 68.8	37.1	9.6–18.4	12.8	2– 8	5.0	ditto
			28	25.6–113.6	69.2	8.8–17.6	11.8	3–11	6.6	Yellow brown, smoky brown, or dark brown
	2	Nutrient	21	25.6– 80.0	47.0	9.6–18.4	13.5	2– 9	5.8	ditto
			28	32.0–112.0	64.6	9.6–13.6	11.0	3–10	7.2	Smoky brown to dark brown
	1	Nut.-dex.	28	46.4–110.4	76.2	9.6–14.4	11.7	5–10	7.5	Smoky brown to dark brown
	All media combined		28	22.4–128.0	64.0	8.8–17.6	11.6	2–11	6.7	
			21	19.2– 80.0	41.2	9.6–18.4	13.1	2– 9	5.3	
Sugar cane	1 (3 cultures)	Sucrose	28	27.2– 80.0	55.3	9.6–16.0	12.1	2–10	6.2	Pale brown
			21	35.2– 92.8	67.2	9.6–17.6	13.1	4–10	7.7	Smoky brown to dark brown
		Corn meal	28	27.2– 76.8	55.3	9.6–16.0	11.6	2– 9	5.8	Very pale brown, almost colorless
		Nutrient	21	36.6– 88.0	62.4	9.6–14.4	11.8	3– 8	6.5	Pale brown
			28	25.6– 70.4	47.6	8.8–16.0	12.1	2– 8	3.8	Very pale brown, almost colorless
		Nut.-dex.	21	25.6– 80.8	49.6	9.6–16.8	12.1	2– 7	3.8	Pale brown to very pale brown
			28	25.6– 78.4	45.6	9.6–12.8	11.2	2– 8	4.1	Pale brown
			21	27.2–108.8	68.1	9.6–17.6	13.4	2–10	6.4	Light brown to dark brown
	All media combined		28	25.6– 80.0	49.9	8.8–16.0	11.7	2–10	4.9	
			21	25.6–108.8	62.0	9.6–17.6	12.7	2–10	6.3	

versely, spores of *H. sacchari* (C) are smaller at 28° than they are at 21° C., except on *standard nutrient* agar, where spore size is little affected by a change in temperature. At 28°, the mean spore length of *H. sacchari* (N) corresponds very closely to the mean spore length of *H. sacchari* (C) at 21° C. Mitra (20) has shown that *H. sacchari,* from cane, produces larger spores at 20° than at 30° C., and this is in agreement with local findings for *H. sacchari* (C).

That such differences can be obtained at different temperatures, emphasizes the importance of recording the temperature at which the fungus was grown when spores of *Helminthosporium sacchari* are being measured.

For some isolates of *Helminthosporium sacchari* (N), temperature had little or no effect on spore color, but for other isolates the spores were darker at 21° than at 28° C. At the higher temperature, several isolates produced spores that were lemon-yellow-brown or light yellow-brown with a greenish tint. Spores of *H. sacchari* (C) were consistently darker at 21° than at 28° C. The yellowish tint noted above for spores of *H. sacchari* (N) was absent in spores of *H. sacchari* (C).

Effect of Isolate

Approximately the same type of growth was obtained when the 3 cultures (single isolate) of *Helminthosporium sacchari* (C) were compared on the same substrate at room temperature (27–29° C.). In marked contrast, considerable variability in type of growth was found within the isolates (usually 7 in number but occasionally 8) of *H. sacchari* (N). On certain media the differences were particularly outstanding. Cultures were held in continuous darkness or in alternating light and darkness. Zonate rings were formed with fluctuating light, absent in continuous darkness (Fig. 4).

Growth. There was little or no difference in the growth of *Helminthosporium sacchari* (N) and the growth of *H. sacchari* (C) on potato-dextrose agar. Growth was very rapid, the fungus producing a thick mat of grayish-green, markedly aerial mycelium. Peripheral growth remained devoid of color until a plate was completely covered. *H. sacchari* (C) grew slightly more rapidly and tended to produce a greener mycelium in continuous darkness than in alternating light and darkness. The under side of an old plate culture was purplish-black, but the medium was not pigmented.

On standard nutrient, *Helminthosporium sacchari* (C) formed a light brown, slightly aerial mycelium (Fig. 4). Some isolates of *H. sacchari* (N), for example the Kawailoa isolate, also shown in figure 3, resembled *H. sacchari* (C) but differed by producing white aerial mycelium at the center and periphery of plate cultures. The Kawailoa type of growth also was obtained with isolates from Ulupalakua (Maui), Waimea (Oahu and Hawaii), East Molokai, and Hanalei (Kauai), as is illustrated in figure 5. The isolate from Mokuleia (Oahu) resembled *H. sacchari* (C) but grew more slowly, while the Kona isolate differed from all other Napier isolates by sporulating vigorously to impart to the culture a bluish-black color.

On corn meal, *Helminthosporium sacchari* (C) formed a sparse, non-aerial, rapidly growing, almost colorless mycelium; 6 isolates of *H. sacchari* (N) grew similarly on corn meal, but the isolates from East Molokai and Hanalei in addition produced aerial mycelia.

On nutrient-dextrose, *Helminthosporium sacchari* (C) produced a luxuriant growth of aerial mycelium, at first colorless, later greenish-gray, and finally brownish-black. White tufts of mycelium were interspersed throughout the culture, or more than one type of growth was obtained, evidence that mutations occur within the species. Submerged hyphae were dark in color, but the medium was not pigmented. Crystals were common in old cultures. The growth of *H. sacchari* (N) was variable on this medium, depending on the isolate studied. In general, four types of growth have been found, namely: (a) Luxuriant, grayish, aerial mycelium with occasional tufts of almost pure white hyphae irregularly distributed; obtained with isolates from Waimea (Oahu and Hawaii), East Molokai, and Ulupalakua. (b) Grayish-white aerial mycelium plus abundant, almost pure white aerial mycelium, the latter not necessarily in tufts, the growth very irregular in radial expansion; obtained with Mokuleia isolate. (c) Grayish-white mycelium, evenly overgrown with abundant white mycelium; obtained with Hanalei isolate. (d) Abundantly produced spores giving a bluish-black color to surface of culture with little or no white mycelium visible until several days after surface of a plate is covered, when a few tufts may appear, particularly at the center of the plate; obtained with Kona isolate.

On 1 per cent sucrose agar, in alternate light and darkness, *Helminthosporium sacchari* (C) formed a zonately ringed growth, which was dark greenish-brown by transmitted light and dark-brown with a greenish tint by reflected light. The type of growth resembled that pictured by Bourne (2) for *H. ocellum* at 33° C. In continuous darkness, there was reduction in growth (Fig. 4); the center of a plate culture was light brown with a greenish tint, while peripheral growth was dark green, almost black, and irregular. The older the culture, the more distinct were the colors.

The Kawailoa isolate of *Helminthosporium sacchari* (N) was the only one studied on sucrose in alternate light and darkness. Growth was at first light-brown soon changing to dark-green, with an irregular, slightly dichotomously branched margin (Fig. 4). Zonate ringing was present but was not pronounced. In continuous darkness, growth of this isolate was more rapid (Fig. 4) and the fungus resembled *H. ocellum* at 31° C., as pictured by Bourne (2). Isolates from Waimea (Oahu) and Ulupalakua grew similarly on sucrose, but with other isolates, 3 additional types of growth were obtained, namely: (a) Mycelium light yellow-green with margin of culture slightly darker; obtained with isolates from Waimea (Hawaii) and East Molokai. (b) Same as Kawailoa isolate, but fungus produced peripheral sectors of grayish-brown mycelium, reminiscent of the growth of the Napier fungus on nutrient-dextrose; obtained with Hanalei isolate. (c) Faster

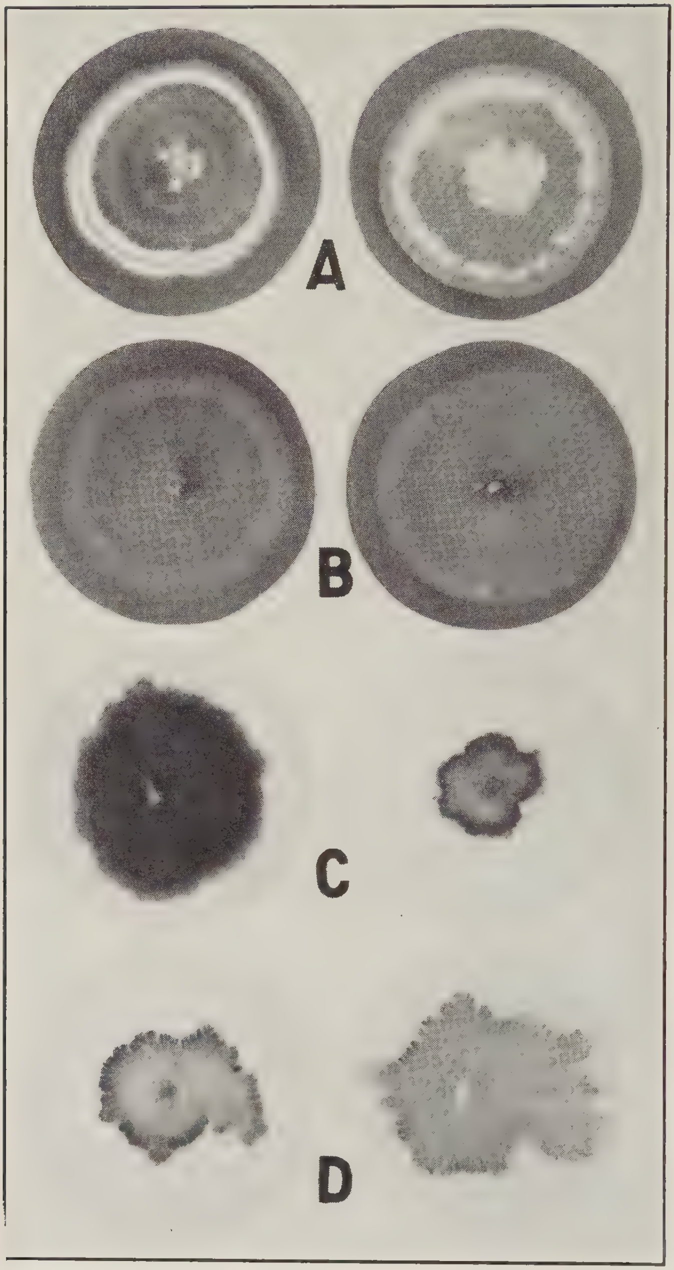

FIG. 4. Effect of substrate and light on growth of *Helminthosporium sacchari* (N), (A and D) and on *H. sacchari* (C), (B and C). A and B on standard nutrient agar; C and D on sucrose agar. Four cultures on left exposed to alternate light and darkness; four on right grown in continuous darkness. Isolate of *H. sacchari* (N) from Kawailoa, Oahu.

growth than obtained with other isolates, darker, with dichotomous branching pronounced; obtained with isolates from Kona and Mokuleia. The last isolate formed some white aerial mycelium (Fig. 5).

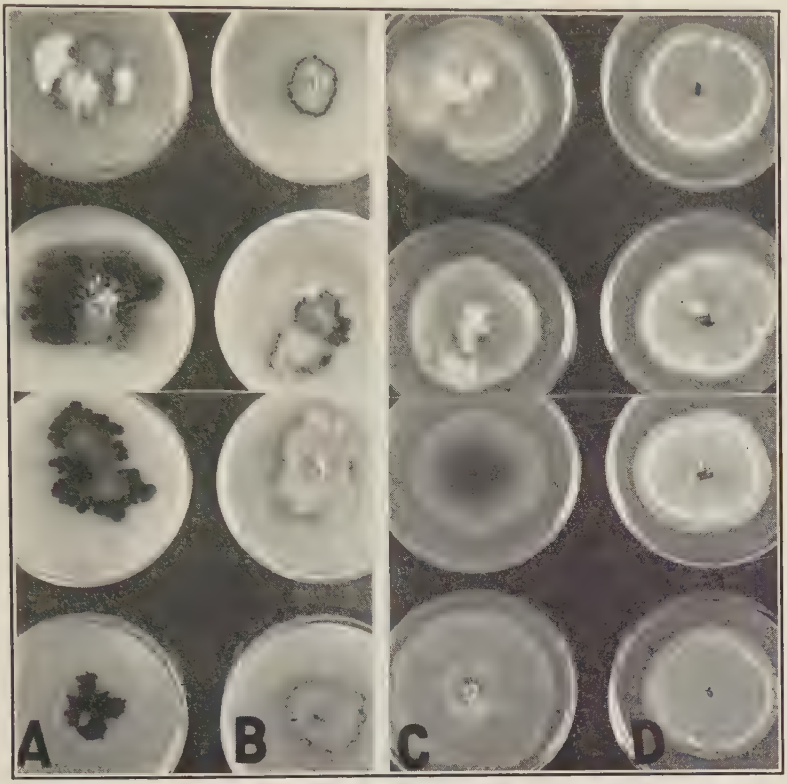

FIG. 5. Cultural variability in 8 isolates of *Helminthosporium sacchari* from Napier grass and cane when grown on sucrose agar (A and B) and standard nutrient agar (C and D). Order in which isolates occur from top to bottom: A and C, Napier from Hanalei, Napier from Mokuleia, Napier from Kona, and cane from Makiki; B and D, Napier from Ulupalakua, Napier from Waimea (Oahu), Napier from Waimea (Hawaii), and Napier from East Molokai.

Substrate had less effect in changing spore size than did temperature. However, spores of *Helminthosporium sacchari* (C) were consistently shorter on nutrient-dextrose than on any other medium. Few to no spores were produced by *H. sacchari* (N or C) on potato-dextrose agar. The Kona and Hanalei isolates were unique in that they sporulated on standard nutrient as well as on corn-meal and on sucrose; the Kona isolate in addition sporulated on nutrient-dextrose, thereby resembling *H. sacchari* (C) more closely than any other isolate of *H. sacchari* (N).

The color of the spores of *H. sacchari* (N) varied from yellowish-brown through smoky-brown to dark-brown, depending on the isolate and the substrate; spores of *H. sacchari* (C) varied from very pale-brown (almost colorless) through smoky-brown to dark-brown.

Effect of Previous Growth Medium

Growth of *Helminthosporium sacchari* (C) and of *H. sacchari* (N)[4] was influenced by the medium from which the inoculum was obtained, particularly when the fungus was grown on 1 per cent sucrose (Fig. 6). If inoculum was taken from standard nutrient, subsequent growth on sucrose was faster than if the inoculum was obtained from sucrose or from corn meal *H. sacchari* (C) produced white tufts of mycelium on sucrose when transferred from nutrient but not when the inoculum was transferred from sucrose or corn meal. These differences were not observed when the fungus was transferred to corn-meal or to nutrient from any other medium.

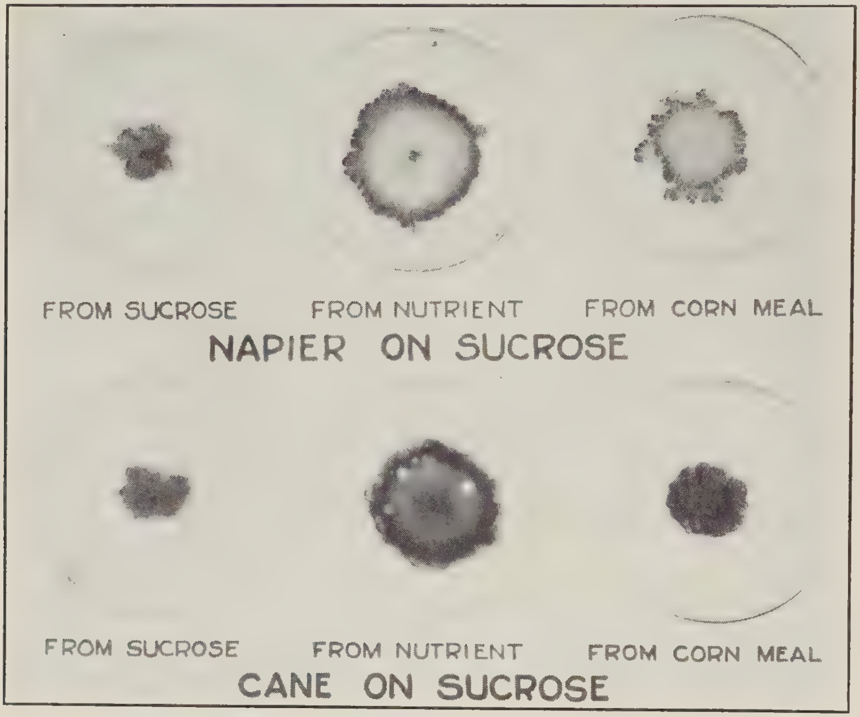

FIG. 6. Effect of previous growth medium on amount and type of growth of *Helminthosporium sacchari* on sucrose agar. Fungus isolated from cane and from Napier (Kawailoa isolate); 8 days' growth at 28° C. in continuous darkness. Note that greatest growth is made when source of inoculum is standard nutrient. Other points of difference are discussed in the text.

As previously mentioned, Mitra (20, 21) and McRae (19) have shown that *H. sacchari* is a highly variable fungus. From the foregoing studies, the writer agrees with these workers, and believes that the differences demonstrated here between *H. sacchari* (N) and *H. sacchari* (C) do not justify a species separation. It is concluded that eye-spot of Napier and eye-spot of sugar cane in Hawaii, and probably elsewhere, are caused by different strains of *H. sacchari*.

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[4] Kawailoa isolate only was subjected to this study.

Pathogenicity

The fungus is easily isolated in pure culture from diseased Napier leaf or stem lesions. Spores were atomized on healthy Napier plants which were then placed in a moist chamber for 12 to 18 hours, removed, and transferred to the greenhouse. Symptoms appeared in 2 to 3 days and closely resembled those observed in the field but seldom attained equal severity. From the artificially induced lesions, the fungus was re-isolated and identified in pure culture, thus completing Koch's rules of proof. Voorhees (29) has also demonstrated the pathogenicity of *Helminthosporium sacchari* (*ocellum*) to Napier.

On varieties of sugar cane susceptible to *Helminthosporium sacchari* (C), strains of *H. sacchari* (N) fail to produce typical eye-spot.[5] Small, linear rather than elongate lesions are formed, and runners are absent. The Napier strains are not regarded locally as a menace to sugar-cane production. The writer has demonstrated that cane varieties resistant to *H. sacchari* (C) are not affected by any of the 10 isolates of *H. sacchari* (N).

Helminthosporium sacchari (C), single isolate only, was tested for its pathogenicity to Napier. The plant is susceptible, but lesions are small and not of economic importance. Merker grass, highly resistant to *H. sacchari* (N), is apparently immune from *H. sacchari* (C).

EPIPHYTOLOGY

Unlike eye-spot on sugar cane, which is most severe in Hawaii during the winter months (16), eye-spot on Napier may be severe at any time of the year. Plants suffering from drought or inadequate irrigation appear to be more susceptible and are killed more rapidly than those receiving adequate moisture.

No effect on the severity of eye-spot has been noted from differential fertilization of Napier with N, P, or K; the disease thus differs from eye-spot of cane, which is severe on plants receiving heavy applications of nitrogen (16).

CONTROL

The disease can be controlled in large measure by replacing Napier with Merker grass or selections from reciprocal Napier × Merker crosses (22). This phase of the problem is discussed in detail elsewhere (23).

SUMMARY

A disease of Napier grass (*Pennisetum purpureum* Schum.) in Hawaii, causing eye-spot of the foliage and cankers on the stems, is described. The disease, which first appeared in October 1939, has caused severe losses; entire plantings of Napier have been rendered useless for fodder and many fields have been plowed and replanted with other grasses.

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[5] Verbal statement to the writer by C. W. Carpenter, plant pathologist of the Hawaiian Sugar Planters' Association Experiment Station, with whose permission the above is released.

The causal agent is *Helminthosporium sacchari* (van Breda de Haan) Butler. Eye-spot of Napier in Florida has been attributed by Stokes and Ritchey (28) and by Voorhees (29) to *H. ocellum* Faris, also the cause of eye-spot of sugar cane, which Mitra (20, 21) and McRae (19) have shown is a synonym of *H. sacchari.* Evidence presented herein confirms this synonymy.

The physiology of 8 isolates of the fungus from Napier and one isolate (3 cultures) of *H. sacchari* from sugar cane was compared. The Napier fungus has an optimum temperature between 21° and 28° C.; the optimum for the fungus from cane has been variously reported as 20°–29° C., 23.5° C., and 30° C. On certain substrates, at uniform temperature, there was little or no difference in appearance of the fungus isolates from Napier and from sugar cane; on other substrates the cane fungus resembled certain Napier isolates and was distinct from other Napier isolates. Within the isolates from Napier, great variation in type of growth occurred on certain media. Spores formed on 4 different substrates at 2 different temperatures (21° and 28° C.) were measured. The fungus isolated from Napier produced larger spores at 28° than at 21° C.; when isolated from cane the spores were larger at 21° than at 28° C. These differences are not believed to be due to different species of *Helminthosporium,* but to different strains of a single species, *H. sacchari.*

The Napier fungus was isolated, inoculated to healthy plants, the disease reproduced and the fungus recovered in pure culture. Inoculated to sugar cane, Napier isolates have little effect and the same is true of *Helminthosporium sacchari* isolated from cane and inoculated to Napier.

Merker grass, a variety of Napier, and certain reciprocal Napier × Merker crosses are highly resistant. The local disease is controlled by substitution of this resistant material for Napier.

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REACTION OF PEA VARIETIES TO SEPTORIA PISI

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(Accepted for publication April 26, 1941)

In 1936, while investigating certain virus diseases of peas (*Pisum sativum* L.) in Colorado, the leaf spot (*Septoria pisi* West.) was noted among certain varieties of foreign origin. It was believed that the initial infection originated from the seed. Throughout the growing season, the disease spread naturally to quite a number of other varieties planted in the same plot causing considerable damage to most of them. Since the preliminary evidence indicated that tolerance or resistance might not be common, a study of varietal reaction to the disease was conducted.

Although this disease has been known for many years and has been reported from nearly all sections where peas are grown, it has never caused serious damage to large acreages of either canning or market garden varieties. From its general prevalence in the experimental plantings and from the damage caused, it seemed likely that the disease could produce considerable destruction to commercial plantings if conditions prevailed that were ideal for the spread and development of the causal organism.

Reports have indicated that the disease is an important factor in plantings of Austrian Winter field peas in certain sections of the country. In 1936, Weimer[1] reported this fungus as being the most generally distributed of any of the fungi attacking peas in Georgia. More recently Sprague,[2] reported that *Septoria pisi* in western Oregon is often the sole cause of severe injury.

The purpose of this paper[3] is to record the varietal reaction of a number of pea varieties to this fungus.

METHODS

These studies were conducted from 1936 to 1939 under field conditions in Colorado. The data collected during the first 2 years of the study were in conjunction with experimental plantings principally designed for an investigation of certain virus diseases of peas. Not all of the varieties were tested every year. In certain years they were replicated; in others they were not. With one exception each variety was tested at least 3 times in 2 years and a few of the varieties in each of 4 years. The number of plants of each variety tested in any one year ranged from 20 to 70 with an average of approximately 35–40 plants.

When the plants were about 4–6 in. tall they were sprayed with a spore suspension of the organism, by using a 2-gallon pressure spray tank.

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[1] Weimer, J. L. Diseases of Austrian Winter peas. U. S. Dept. Agr., Bur. Pl. Ind., Plant Disease Rptr. 20: 210–212. 1936.

[2] Sprague, R. Notes on Septoria scald on vetch and peas in Oregon. Phytopath. 30: 541–542. 1940.

[3] The writer expresses his appreciation to E. C. Tatman and V. R. Boswell for advice and assistance in the statistical analysis of the data.

Infected pea straw was used as the source of inoculum. The straw was immersed in water for a short period to liberate the spores. The spore suspension was then strained through cheesecloth and used to spray the young pea seedlings. The inoculation was performed in the evening in order to retard as much as possible the evaporation of the moisture from the inoculated plants. A second inoculation was made about 2 weeks following the first in order to be assured of a uniform infection. About 3 weeks after the first inoculation, wide-spread infection was noted on the lower leaves of many varieties. Frequent showers and, in certain years, an occasional light hail were in a large measure responsible for the amount and rate of infection.

Infection ratings were made 3 times throughout the season. At the end of approximately 10 weeks, plants showing mild infection were given a rating of 1 to 2, moderate infection 2 to 3, severe infection 3 to 4, and very severe infection 4 to 4+. The latter included plants that had died as a result of the disease.

The data were analyzed according to Fishers' method for the analysis of variance. Owing to inconsistencies in the experimental design, it was impossible to set up a single analysis which would cover the results of all the tests. Hence the data were analyzed as several smaller tables, each covering certain varieties tested in 2 or more years in single or replicate tests.

SYMPTOMS

The initial symptoms were noted as yellowish-green indefinite areas on the leaves, which gradually darkened and enlarged until the entire leaflet was involved. Later the petioles and stipules became infected, followed by the nodes which became shrunken and sometimes girdled. In the lesions, pycnidia developed within a few weeks following infection. When infec-

TABLE 1.—*Summary of variance analyses of the reaction of 131 pea varieties and strains to Septoria pisi*

Number of varieties and strains compared	Years compared	Blocks	Differences due to[a]	
			Years	Varieties
4	1936, 1937, 1938, 1939	1	–	–
10	1936, 1937, 1939	1	–	++
5	1937, 1938, 1939	1	–	–
8	1936, 1938, 1939	1	–	+
5	1936, 1938, 1939	2	–	++
71	1936, 1937, 1938	1	–	++
6	1936, 1939	2	–	++
9	1936, 1939	1	–	++
13	1937, 1939	1	–	+
42	1938, 1939	2	+	++
81	1936, 1937	1	–	++
97	1937, 1938	1	–	++
53	1936, 1938	2	–	++
78	1936, 1938	1	–	++

[a] Minus sign (–) represents non-significant; (+) significant; (++) highly significant.

TABLE 2.—*Reaction of peas to Septoria pisi*

Variety	Time of maturity[a]	No. years tested	Number of tests	Disease index mean and S.E.
I. Canning Varieties				
Alaska 2B	E	2	3	4.0 ± .27
Alaska, Asgrow	E	3	5	3.9 ± .32
Alaska Large Podded	E	3	4	3.9 ± .36
Alaska, Maryland	E	3	4	4.2 ± .36
Asgrow Canner King	M	2	4	2.7 ± .19
Asgrow Early Harvest	E	2	3	3.8 ± .27
Asgrow Pride	M	2	3	2.3 ± .27
Asgrow Triumph	M	2	3	3.3 ± .27
Bruce	L	3	5	2.8 ± .32
Canners Delight	L	2	4	1.4 ± .19
Canners Gem	M	3	5	2.2 ± .32
Canners Gem	M	2	3	2.7 ± .27
Chief	M	2	3	2.8 ± .27
Climax	M	2	3	2.3 ± .27
Green Admiral	L	2	3	3.0 ± .27
Green Admiral	L	4	6	2.8 ± .57
Green Giant	L	3	5	2.7 ± .32
Horal	L	3	4	2.8 ± .41
Horsford	L	3	5	2.2 ± .32
No. 58	L	2	3	2.3 ± .27
Major	L	2	4	3.0 ± .19
Market Surprise	E	2	3	4.0 ± .27
Meteor	E	3	5	3.9 ± .32
Perfectah	L	3	5	2.6 ± .32
Perfection	L	3	5	1.1 ± .18
Perfection	L	3	4	2.0 ± .36
Perfection	L	3	5	1.8 ± .32
Perfection, Early	M	2	3	1.7 ± .27
Perfection, Wisconsin	L	3	4	2.2 ± .36
Perfection, Wisconsin	L	3	5	1.7 ± .32
Premium Gem	E	3	5	3.4 ± .32
Premium Gem	E	2	3	2.5 ± .27
Prince of Wales	L	3	4	2.7 ± .36
Profusion	L	3	5	2.9 ± .32
Rice No. 13	L	4	6	4.1 ± .33
Rogers Ace	E	2	4	3.1 ± .19
Rogers Delicious	L	2	4	1.6 ± .19
Rogers Famous	L	2	4	1.9 ± .19
Rogers Gem	M	2	3	2.0 ± .27
Rogers Kay	L	2	4	3.6 ± .19
Senator	L	3	5	2.4 ± .32
Senator	L	2	3	3.0 ± .27
Short Admiral	L	2	3	2.5 ± .27
Stella	E	3	3	4.0 ± .36
Surprise	E	3	5	4.0 ± .32
Winner	E	3	5	3.7 ± .32
Wisconsin Early Sweet	E	3	5	4.0 ± .32
Yellow Admiral	L	3	5	2.6 ± .32
Yorkshire Hero	L	2	4	2.1 ± .19
II. Market Garden Varieties				
Admiral Beatty	L	2	4	3.4 ± .19
Alderman	L	3	5	3.0 ± .36
Alderman	L	3	4	3.0 ± .32
American Wonder	E	3	5	3.6 ± .32
Asgrow No. 40	L	3	5	3.0 ± .32
Banqueteer	E	3	4	3.9 ± .36
Bliss Everbearing	L	2	4	2.5 ± .19

TABLE 2.—(*Continued*)

Variety	Time of maturity[a]	No. years tested	Number of tests	Disease index mean and S.E.
II. Market Garden Varieties—(*Continued*)				
Blue Bantam	E	3	6	3.0 ± .26
Bountiful	E	3	4	3.8 ± .36
British Lion	E	3	4	3.9 ± .36
Carter Eight Weeks	E	3	5	3.8 ± .32
Charles 1st	M	2	3	2.5 ± .36
Confidence	M	2	4	3.5 ± .19
Dark G.O.P.	L	3	3	2.2 ± .40
Duplex	L	3	4	3.3 ± .36
Dwarf Alderman	L	2	3	2.5 ± .27
Dwarf Champion	L	2	3	2.3 ± .27
Dwarf Telephone	L	3	5	2.5 ± .32
Dwarf Telephone	L	3	4	2.7 ± .36
Early Eight Weeks	E	2	4	3.9 ± .22
Early Gilbo	L	3	4	3.0 ± .36
Everbearing	L	3	5	2.4 ± .37
Glacier	E	2	4	3.8 ± .19
Gradus	E	3	5	3.6 ± .32
Gradus	E	2	3	3.0 ± .27
Horsford	L	3	5	2.2 ± .28
Hundredfold	M	3	5	3.5 ± .32
Hundredfold Improved	M	2	2	3.0 ± .38
Hurst Monarch	M	2	4	3.9 ± .19
Kelvendon Wonder	E	3	5	3.7 ± .24
Laxton Progress	E	3	5	3.9 ± .32
Laxton Progress	E	2	3	4.1 ± .27
Laxton Progress	E	3	5	3.8 ± .32
Laxton Superb	E	3	6	3.6 ± .26
Laxtonian	E	2	4	3.0 ± .19
Lincoln	L	3	5	2.2 ± .32
Little Marvel	E	3	5	3.4 ± .32
Lord Chancellor	M	3	3	2.2 ± .36
Nott Excelsior	E	3	5	3.5 ± .32
Onward	L	3	5	2.1 ± .32
Onward	L	2	4	2.9 ± .19
Pedigree Extra Early	E	3	5	4.1 ± .32
Peter Pan	M	2	4	2.7 ± .19
Pilot Improved	E	3	4	4.2 ± .36
Pioneer	E	3	5	3.3 ± .32
Potlatch	L	3	4	2.2 ± .32
President Wilson	M	4	7	3.6 ± .31
Pride of Market	L	3	5	2.1 ± .28
Prolific Early Market	E	2	3	4.0 ± .27
Quite Content	M	3	5	3.2 ± .32
Rogers Giant Podded Hamper	L	2	4	2.9 ± .19
Rogers No. 93	L	3	4	2.5 ± .32
Rogers No. 95	L	2	4	1.9 ± .19
Sharp Miracle	E	2	3	3.7 ± .27
S.S. Pioneer	M	3	4	3.1 ± .31
Stratagem	L	3	4	2.2 ± .43
Stratagem	L	3	5	2.6 ± .32
Sutton Excelsior	M	3	5	3.3 ± .32
Sutton Foremost	E	2	4	4.1 ± .31
Teton	E	2	4	4.1 ± .19
Thomas Laxton	E	3	5	4.0 ± .32
Tom Thumb	L	2	4	2.4 ± .19
World Record	E	2	3	4.2 ± .27
Zwaan Paramount	L	3	4	2.9 ± .31

TABLE 2.—(*Concluded*)

Variety	Time of maturity[a]	No. years tested	Number of tests	Disease index mean and S.E.
III. Edible Podded Varieties				
Dwarf Gray Sugar	M	3	5	2.7 ± .32
Dwarf Gray Sugar	M	3	5	3.1 ± .32
Dwarf White Sugar	M	2	3	2.5 ± .27
Mammoth Melting Sugar	L	3	5	2.7 ± .32
Mammoth Pod Early	E	2	3	4.2 ± .27
Moerheim Giant	L	2	3	3.2 ± .31
Paramount Sugar	M	3	5	3.0 ± .32
IV. Field Varieties				
Austrian Winter	L	3	5	2.5 ± .32
Black Eye Marrowfat	L	3	4	1.6 ± .36
Blue Prussian	L	4	7	1.8 ± .43
Capucijner	L	2	3	2.8 ± .27
Harrison Glory	L	3	4	2.4 ± .36
Harrison Glory	L	2	3	2.5 ± .27
Harrison Glory	L	3	5	3.0 ± .32
Imported (from China)	L	2	4	2.9 ± .19
Imported (from Puerto Rico)	L	2	4	1.1 ± .19
Kootenay	L	3	4	3.2 ± .31
Maple	L	3	5	2.2 ± .32
Swedish Yellow	L	3	4	3.1 ± .36
White Canada	L	3	5	3.1 ± .32
White Eye Marrowfat	L	3	5	2.4 ± .37

[a] E, represents early; M, medium; L, late.

tion took place early, young plants of very susceptible varieties died before producing pods. If the infection was not so serious or occurred when the plants were more mature, a small seed crop was produced.

EXPERIMENTAL RESULTS

The variance analyses showed that, in general, there was no significant difference due to years in which the data were collected, except in one instance (Table 1). There were, however, significant differences due to varieties in all except two tests, which were made up of 4 and 5 varieties, respectively. A summary of these analyses is recorded in table 1.

The canning varieties with their respective mean reaction to the disease are listed in table 2, which shows that only a few varieties exhibited any appreciable degree of resistance. Of the varieties tested, 7 were mildly, 20 moderately, and 22 severely infected. Most of the varieties showing mild infection were of the Perfection type. In addition to these, Canners Delight, Rogers Delicious, and Rogers Famous were also quite tolerant.

None of the market garden varieties exhibited as high a degree of tolerance as the most tolerant canning varieties (Table 2). Of the 64 market garden varieties tested, only one, Rogers No. 95, showed mild infection, 24 were moderately infected and 39 exhibited severe and very severe infection.

None of the edible podded varieties (Table 2) was highly tolerant, but only one, Mammoth Pod Early, was extremely susceptible.

Of the field varieties tested (Table 2) three were mildly infected, seven moderately, and four severely. An import from Puerto Rico exhibited the greatest resistance of any of the varieties tested.

DISCUSSION

It is evident that among the 134 varieties and strains of peas tested, only 2 showed promise from the standpoint of high tolerance to *Septoria pisi*, namely one strain of Perfection and an unnamed import from Puerto Rico. The latter is a very late maturing variety of poor type, whereas the former belongs to a group of important canning varieties. It is possible that either of these strains would be suitable for parental material if hybridization studies for disease resistance were conducted.

There seems to be a relationship between earliness of maturity and extreme susceptibility and also between moderate tolerance and later maturity. Among the 42 early varieties tested in all of the classes, 41 were either severely or very severely infected and one was moderately infected. Table 3 shows that the group mean reaction of these varieties was 3.9. Among the 24 medium maturing varieties, one was mildly infected, 12 moderately, and 11 severely, and the group mean reaction was 2.8 (Table 3). Of the 68 late varieties, 9 were mildly infected, 42 moderately, 16 severely, and 1 very severely. The mean reaction of this group was 2.6 (Table 3). From this it appears that there is no significant difference between the mean reactions of the medium and the late maturing groups of varieties, but that the difference between the early maturing and the other two groups appears to be significant.

TABLE 3.—*Differences in average mean reaction to Septoria pisi between early, medium and late maturing pea varieties*

Type of pea	Mean disease index for number of varieties of the season of maturity shown							
	Early		Medium		Late		Total	
	No.	Index	No.	Index	No.	Index	No.	Index
Canning	14	3.7	9	2.4	26	2.5	49	2.9
Market Garden	27	3.7	11	3.1	26	2.6	64	3.1
Edible Podded	1	4.2	4	2.8	2	2.9	7	3.3
Field	......	......	......	......	14	2.4	14	2.4
Total	42	3.9	24	2.8	68	2.6	134	2.9

SUMMARY

One hundred and thirty-four strains and varieties of peas were inoculated with *Septoria pisi* under field conditions from 1936 to 1939, in order to determine their relative resistance and susceptibility. Only two varieties exhibited a very high degree of tolerance. The canning varieties of the Perfection type were in general the most tolerant group. In general the earlier varieties were the more susceptible ones and the later varieties the more tolerant ones.

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ANGULAR LEAF SPOT OF MUSCADINES, CAUSED BY MYCOSPHAERELLA ANGULATA N. SP.[1]

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INTRODUCTION

Muscadine grapes (*Vitis rotundifolia* L.) have not been the subject of careful pathological investigations in the past, hence they are generally regarded as being relatively disease-free.

Studies by the writer, extending over the past 3 years, have brought to light several destructive foliage diseases and berry rots that either have escaped the attention of pathologists or have been assumed to be the same diseases, generally well known on bunch grapes.

Following is a report on the symptomatology and etiology of one of the foliage diseases caused by a pathogen known heretofore as *Cercospora brachypus* Ell. and Ev. (1). Because of the characteristic shape of the lesions on most varieties of muscadines, angular leaf spot of muscadines seems an appropriately descriptive name for the disease.

SUSCEPTS AND RANGE

Field observations and inoculation experiments under controlled conditions indicate that all the common varieties of muscadine grapes are susceptible to angular leaf spot, although not necessarily to the same degree. In general, the older varieties, such as Scuppernong, Flowers, and Thomas, apparently suffer less than the more recent introductions of better quality, as Hunt, Yuga, Creek, Stuckey, Howard, etc. Likewise, field observations and inoculation trials have given no indication that several common varieties of bunch grapes, such as Concord, Delaware, etc., are susceptible to muscadine angular leaf spot. In certain large vineyards in the vicinity of Experiment, Georgia, as well as in several home plantings of a few vines, in which muscadine and bunch grapes have been growing side by side for years, muscadine angular leaf spot could be found only on the muscadine varieties.

Since there has been no literature published on this disease, other than a brief description of the imperfect stage of the pathogen (1, 5), one can do no more than guess as to its range. It might be assumed, however, that the disease is coextensive with muscadine culture. It is certainly the dominant leaf spot on muscadines in the vicinity of Experiment, Georgia; and, since the type material was collected in Alabama, it might safely be assumed that it is present throughout the southeastern States.

SYMPTOMATOLOGY

The earliest evidence of infection appears as small chlorotic areas, most noticeable on the upper leaf surface. Following this, the individual lesions develop fairly rapidly and soon a small, dark brown to black, necrotic area appears in the center of each of them (Fig. 1, A, B, C). Continued development results in the formation of angular to irregular lesions, discrete, for the

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[1] Paper No. 83, Journal Series, Georgia Agricultural Experiment Station.

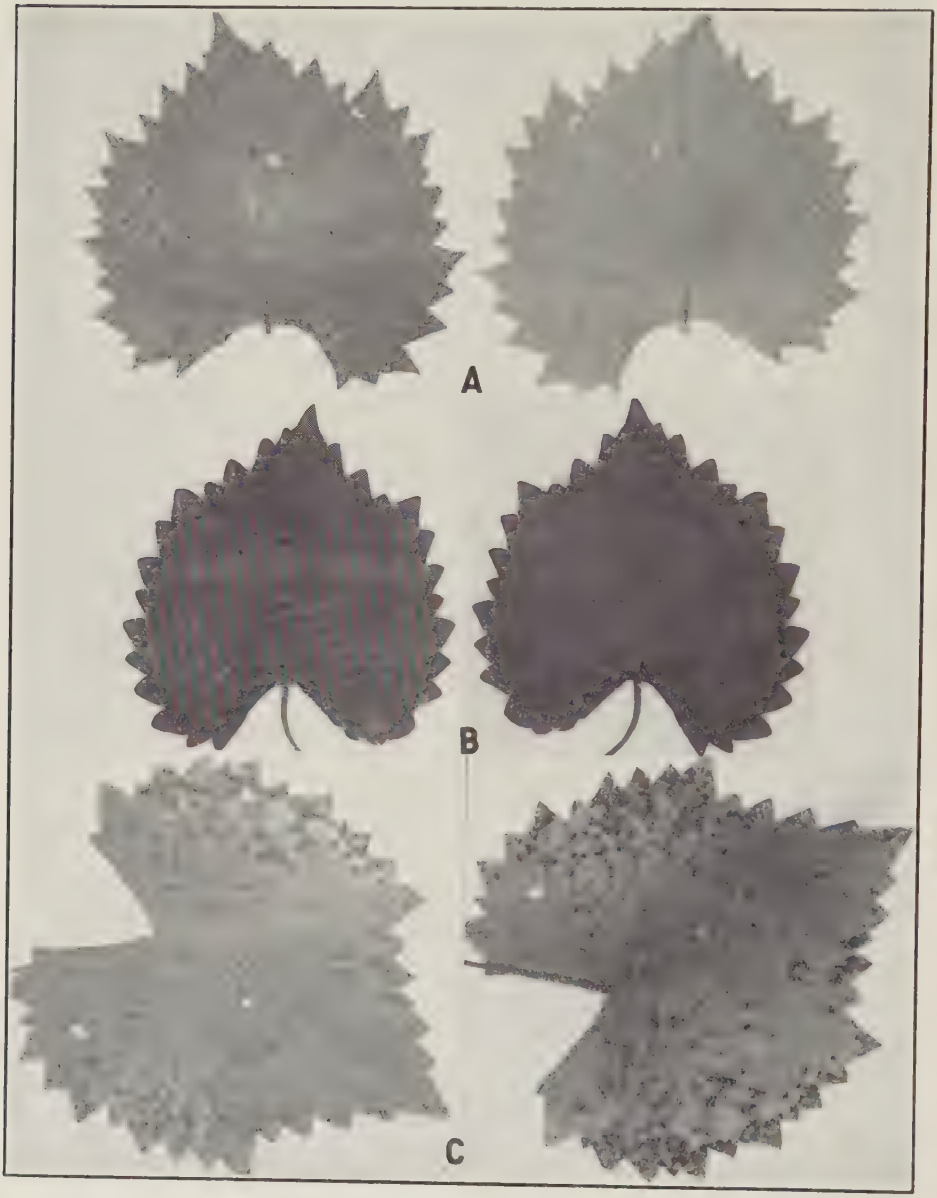

FIG. 1. Photographs showing the symptoms of angular leaf spot of muscadines as they appear on the upper (left) and lower (right) leaf surfaces of several varieties of muscadines. A. on the Hunt; B. on the Yuga; C. on an unnamed male, older lesions. A single lesion of muscadine black rot is present on the Hunt (lower left and right side, respectively). Note the presence of halos on the young lesions. Since the photographs were taken with transmitted light, the halos tend to be evident on the lower surfaces as well as on the upper. × ½.

most part, and tending to be confined to the areolae of the leaf. At first, distinct halos are present, but these tend to disappear with age. On certain varieties—Hunt and Scuppernong—the lesions tend to become more circular (Fig. 1, A), but, when viewed by transmitted light, the angular outline of the primary lesion is readily apparent. Some coalescence of older lesions occurs on most varieties, but it is usually incomplete, so that most of the original lesion is distinct. At maturity the lesions vary in diameter from a few millimeters to several centimeters.

On the lower surface the halos are indistinct to absent from all lesions. The angular necrotic areas are dark brown to black and appear papillate under magnification, due to the presence of numerous small conidiophore tufts. These tufts are numerous on the upper leaf surface only after the lesions are old, but may appear sparsely at other times. In seasons of high humidity, the conidiophores are produced amphigenously on the older lesions, and are light olive-gray to dark olive-gray. The secondary infection cycles continue to operate throughout the season, but the disease becomes epiphytotic rather late, so that the principal effect is that of premature defoliation.

During relatively normal seasons, conidia are not abundant on field material, but, when leaves containing mature lesions are enclosed over night in moist chambers, abundant conidia are produced on both surfaces of the lesions. Under these conditions of high humidity, the conidiphores tend to become flaccid and these, in conjunction with other hyphae growing out from the lesions, present a byssoid appearance, particularly on the lower leaf surface.

ETIOLOGY

A fungus causing a spotting of the leaves of muscadines was collected near Tuskegee, Alabama, by G. W. Carver and sent to Ellis, who, in 1902, described the fungus as *Cercospora brachypus* Ell. and Ev. Since, to the writer's knowledge, this is the only species of *Cercospora* that has been described on muscadines, he was reasonably certain that his collections represented Ellis' fungus. However, since the original description is so brief and no information has been published subsequently, field collections were sent to several taxonomists for comparison with the type material.[2] The general consensus is that the present material is identical with *Cercospora brachypus* Ell. and Ev., although Ellis' material shows larger and more circular lesions than did the material submitted by the writer. Since all other details, however, are in agreement, and particularly since the writer has since found considerable variation in the size and conformation of lesions on various varieties of muscadines, it is felt that the above mentioned discrepancy is not taxonomically significant.

A study of the life cycle of the pathogen has shown that in addition to the imperfect stage, the fungus develops spermogonia and perithecia in overwintering leaves, and, as the perfect stage is an undescribed *Mycosphaerella,* it will hereafter be referred to as **Mycosphaerella angulata** n. sp.

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[2] Acknowledgment is made to Charles Chupp, J. A. Stevenson, and F. J. Seaver for their expressions of opinion as to the identity of the pathogen.

DEVELOPMENTAL MORPHOLOGY

Evidence secured from numerous controlled inoculations using both conidia and ascospores indicates that infection may occur through either leaf surface, and that penetration is both direct and through the stomata. The infection hypha is first intercellular, but, as the host cells die rapidly in advance of the advancing mycelium, an intracellular relationship is established early. No evidence of haustoria has been found. The conidiophore tufts first develop on the lower leaf surface, as stated above, but often become amphigenous with age and high humidity. They originate from subcuticular or subepidermal hyphae and usually begin to produce conidia shortly after they emerge from the leaf surface, long before they assume the characteristic complexity of structure commonly assigned to them. As they grow older, the basal mass is built up into a definite stroma with short emergent fertile hyphae (Fig. 2, A, B).

With age and subsequent cyclic development, spermogonia and perithecia often develop in these old conidiophore bases. Undoubtedly the unsuspected presence of early stages of these reproductive bodies influenced Ellis to describe the conidiophore bases as "sphaeriform." The basal portions of the conidiophores quickly become pigmented, while the aerial portions range in color from almost hyaline to dark olive-gray. Under fairly dry conditions, the fascicles resemble short tufts, the conidia are not abundant, while, under conditions of fairly high humidity, the conidiophores become byssoid in appearance. In the latter case, conidia are abjointed from hyphae that resemble vegetative mycelium, except for the presence of geniculations (Fig. 2, D).

The conidia are hyaline, or nearly so, slender, curved, more or less acute at each end, 1–5 septate, with usually two oil droplets in each cell and measure 16.8–112 × 2.24–3.5 μ. The length and number of septations are influenced by relative humidity. Under average field conditions the conidia measure rather uniformly 47.6–72 × 2.8–3.5 μ. It would thus appear that measurements based on width are more reliable taxonomically than are those based on length.

Conidia germinate uniformly within 3 to 8 hours on a 2 per cent tap agar, when conditions of temperature and oxygen balance are favorable. The germ tubes emerge from the terminal cell at either end or both ends of the spore, and often from other cells, as well.

As stated above the spermogonia often develop within the stromata comprising the conidiophore bases. They may also develop concurrently from more recent infections that apparently never have produced conidia. Spermogonia, like perithecia, have not heretofore been reported for the parasite under consideration, but since the writer and others (3, 4, 2) have repeatedly reported on the development of these structures in the course of studies on other fungi, no great effort has been made toward a critical study of the spermogonia of *Mycosphaerella angulata.* They are initiated in late September and early October; and, while a majority of them mature in October and early November, various developmental stages may be found as late as early spring on the overwintering leaves. They originate in either leaf surface, but are perhaps more often epiphyllous. The details of development are almost identical with those described in other studies (3, 4), and, at maturity, the spermatia occur in groups of 3 or 4 and are liberated through sterigma-like processes from the spermatiferous cells. Mature spermatia are rod-shape, uninucleate, and measure 2–4 × 0.5–0.7 μ.

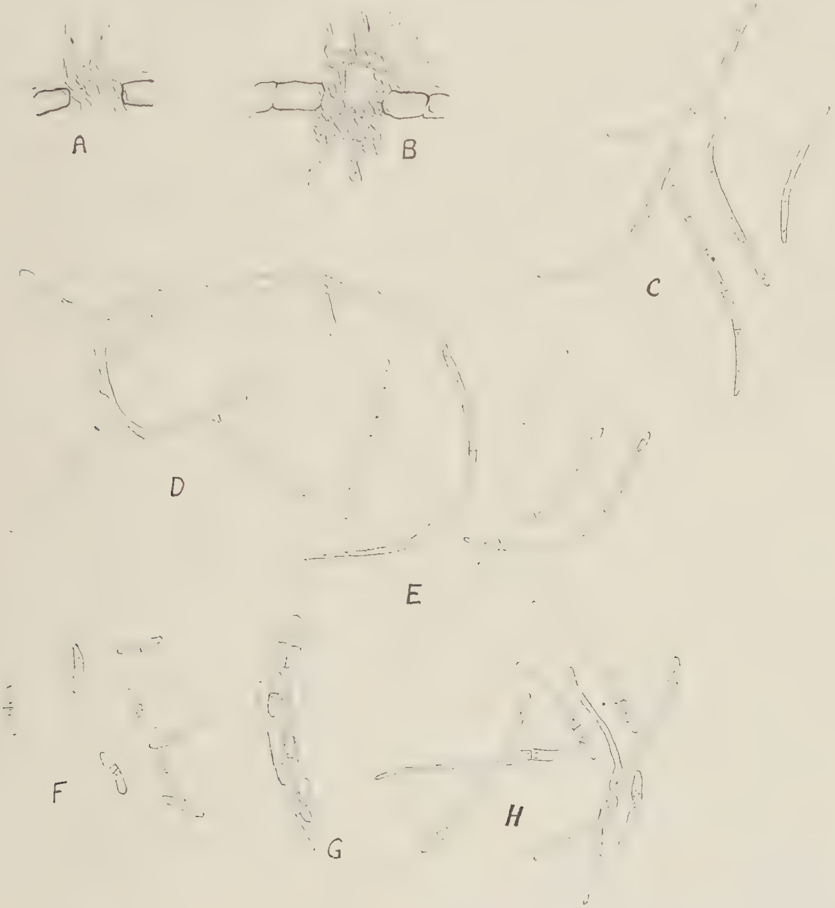

FIG. 2. Spore forms of *Mycosphaerella angulata.* A. Young conidial stroma before a sphaeriform base is evident. B. An older conidiophore fascicle, showing a well-developed basal stroma in which a spermogonium is developing. C–E. Conidia: C, from ascospore culture on 2 per cent potato-dextrose agar; D, from field material left in a moist chamber overnight (note the byssoid conidiophores); E, from field material. F–H. Ascospores: F, appearance when first discharged on agar; G, a mature ascus showing its bitunicate nature; H, germinating ascospores. (All sketches made with the aid of a camera lucida. × 750.)

Perithecial development is initiated concurrently with the spermogonia and in similar positions in the leaf tissue. Those originating outside conidiophore bases frequently begin their development from subcuticular hyphae. Soon one to several deep-staining archicarps, each with an elongate trichogyne and an uninucleate basal cell, become evident. At this stage of development perithecial fundaments are readily distinguishable from young spermogonia.

Due to certain peculiarities in time and mode of development, which have increased certain technical difficulties, a full report on the cytological development of the perithecia will be deferred until a later date. However, it can be stated at this time that the evidence is strong that the spermatia function as male sexual elements and that spermatization is essential to the initiation and maturation of ascospores.

When the perithecia are mature, the asci discharge their spores readily on slight drying. The asci do not mature at one and the same time within a given perithecium, so that the source of primary inoculum is present for some time in the field during the spring. Climatic conditions, particularly showers and subsequent drying, greatly influence the span of the primary cycle.

DEVELOPMENT IN CULTURE

Single and multiple conidial isolates were obtained by streaking spore suspensions on the surface of hard tap agar, while ascospore isolates were obtained by allowing the spores to discharge upward and stick to the surface of inverted agar plates. By the latter method, single or multiple spore isolations could be had at will, free from contamination, by regulating the distance between the perithecia and the surface of the agar plates. Mature spores were measured both in crushed mounts and immediately after their discharge into the agar plates. When the conditions of elevation and humidity are favorable, one can cause a majority of the mature asci within a perithecium to discharge their spores into the agar within 10 to 15 minutes after setting the spore traps. Under these conditions the ascospores are hyaline, bicellular, straight to slightly curved, guttulate, and measure $14–19 \times 2.8–5.6\ \mu$ (Fig. 2, F, G).

For purposes of comparison, only modified potato-dextrose and malt agars were used. Initial growth and pigmentation were the same on both agars, but isolates from both ascospores and conidia continued to sporulate longer on the malt agar.

Both types of spores germinated readily in from 3 to 8 hours and produced visible growth in about 3 days. In all measurable respects, cultures from conidia and ascospores are identical. When first visible, the mycelium is pale gray to almost white, but after the colonies are several millimeters in diameter, the color ranges from light olive-gray to dark olive-gray; essentially all the color being in the vicinity of the substrate. Isolates from both sources sporulate abundantly within three to five days, but sub-cultures from both types of cultures tend to remain sterile. In this work no great care was taken to sub-culture spores, only, and it may be that such cultures would continue to sporulate whereas sub-cultured mycelium does not. Conidia produced on ascospore isolates are identical with those produced on conidial isolates, and both in turn are identical with conidia produced on field material under similar conditions of temperature and moisture (Fig. 2, C, D, E). Unlike isolates of other Mycosphaerellas reported earlier (2), cultures of *M. angulata* do not undergo much color change even after months on agar, and never become generally stromatic throughout. Very few spermogonia have been found in culture on the media used, and no evidence of perithecia has been seen.

Both conidia and ascospores were used in the inoculation trials. Conidial inoculum was obtained direct from field material, from conidial isolates, and from ascospore isolates; whereas ascospores were obtained from field material, as described above. In all cases resultant infections were identical. The first recognizable infections appeared in the greenhouse 10 days to 2 weeks following inoculation, while the checks remained free from infection in all cases. The above evidence, taken as a body, is convincing proof that the 3 spore forms that have been considered are, genetically, but phases in the reproductive cycle of one and the same organism.

TAXONOMY

The form and development of the perithecia, the asci produced in fascicles, the absence of paraphyses, and the bicellular, hyaline spores are clearly characteristic of the genus *Mycosphaerella* Johans. Since no other *Mycosphaerella* has heretofore been reported on the foliage of *Vitis rotundifolia* L., and since there is no evidence to indicate that any of the varieties of bunch grape are susceptible to the pathogen under consideration, there can be little doubt that this is an undescribed species. It is, therefore, designated **Mycosphaerella angulata** n. sp.[3] with the following diagnosis:

Mycosphaerella angulata n. sp.

Syn: *Cercospora brachypus* Ell. and Ev. (Jour. Mycol. 8: 71, 1902).

Perithecia scattered, mostly in lesions, amphigenous, partly embedded in host tissue, erumpent, ovate to nearly globose, beaked prior to maturity, 40–90 × 40–60 μ, black, ostiolum papillate when mature; asci cylindrical club-shape, short stipitate, fasciculate, 36.4–42 × 8.4–14 μ, aparaphysate, bitunicate, eight-spore; spores uniseriate to imperfectly biseriate in the ascus, bicellular, straight to slightly curved, hyaline, guttulate, 14–19.6 × 2.8–5.6 μ (average 16.8 × 4 μ).

Hab. In overwintered lesions produced by the conidial stage on leaves of *Vitis rotundifolia* L., Experiment, Ga., maturing during spring.

Spermogonia: Scattered in and along margins of lesions produced by the conidial stage, ovate to globose, black, amphigenous but perhaps more often epiphyllous, embedded in leaf tissue but later erumpent, ostiolate, 30–60 × 30–50 μ; spermatia small, rod-shape, hyaline, 2–4 × 0.5–0.7 μ, arising endogenously, usually in fours within spermatiferous cells and liberated through sterigma-like processes.

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[3] Having reference to the shape of the lesions it produces on the foliage of most varieties of *Vitis rotundifolia* L.

Hab. On recently fallen leaves, maturing throughout period from October through February.

Conidial stage: Lesions mostly angular, often confluent, varying in size, 1 mm. to several cm., dark-brown to almost black, surrounded on upper surface by a distinct halo when young, confined to leaves; conidiophores mostly hypophyllous, becoming amphigenous with age, base becoming stromatic, fasciculate to lax, geniculate, pigmented at base, continuous to one to several septae, mostly short; conidia colorless or nearly so, very slender, cylindrical, acute at each end, curved, 16.8–112 × 2.24–3.5 μ (mostly 47.6–72 × 2.8–3.5 μ), 1–5 septate, guttulate, length and septation influenced by humidity.

Hab. Parasitic on leaves of *Vitis rotundifolia* L., causing angular leaf spots and contributing to premature defoliation.

Peritheciis sparsis, plerumque in maculis, amphigenis, semi-immersis, punctiformibus, ovatis vel globatis, 40–90 × 40–60 μ, rostratis cum immaturis, nigris, ostiolis papillato praeditis; ascis cylindraceis clavatis, brevisme stipitatis, fasciculatis, aparaphysatis, bitunicatis, octosporis, 36.4–42 × 8.4–14 μ; sporiidis uniseriatis vel biseriatis, bicellularibus, vix curvatis, hyalinis, guttulatis, 14–19 × 2.8–5.6 μ, plerumque 16.8 × 4 μ.

Hab. in foliis dejectis *Vitis rotundifoliae*, Experiment, Ga.

Spermogoniis autumno efformatis, sparsis, plerumque in maculis et marginatis, ovatis vel globatis, nigris, amphigenis plerumque epiphyllis, innato- erumpentibus, punctiformibus, 30–60 × 30–50 μ; spermatiis bacillaribus, hyalinis, 2–4 × 0.5–0.7 μ.

Hab. in foliis dejectis *Vitis rotundifoliae.*

Statu conidico in maculis angularibus v. irregularibus, confluentibus, rubroferrugineis v. nigris, pallide marginatis, foliis, efformato; hyphis fertilibus plerumque hypophyllis, rare amphigenis, a stromate orientibus, fasciculatis v. byssoideis, geniculatis, hyalinis v. dilute olivaceis-griseis, continuis v. pleuriseptatis, brevisme; conidiis hyalinis, rare sub-hyalinis, angustio-cylindraceis, utrinque acutis, curvulis, 16.8–112 × 2.24–3.5 μ (plerumque 47.6–72 × 2.8–3.5 μ) 1–5 septatis, guttulatis.

Hab. in foliis vivis *Vitis rotundifoliae.*

For the convenience of plant pathologists and mycologists, material has been deposited in the following herbaria: Mycological Collections of the Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C., Farlow Herbarium; Plant Pathology Department, Cornell University, Ithaca, N. Y., and Herbarium of the Georgia Experiment Station, Experiment, Ga.

CONTROL

Results obtained from a single season's work indicate that angular leaf spot of muscadines may be effectively controlled. In this work a 4–5–50 Bordeaux spray was applied at intervals of about 2 weeks, and so timed that the first application preceded the first heavy ascospore discharge in the spring. The exact date for this application was obtained by a close check on the state of development of the perithecial material in the field, and when possible to do so, each subsequent spray was applied just preceding a rain.

Spraying may be discontinued after cessation of the 4–6 weeks' ascospore discharge.

The writer has no information on the degree of control possible through strict adherence to sanitary vineyard practices, but, knowing the nature of the pathogen's life cycle, one would be justified in recommending the destruction, either by fire or deep plowing, every fall, all leaves throughout the vineyard. If the leaves are turned under, one should be careful to avoid uncovering them while planting a cover crop. In the experience noted above, only 4 sprays were used, but the schedule was designed for muscadine black rot, the pathogen of which apparently has a less extended period of ascospore discharge in the spring than does *Mycosphaerella angulata.*

DICUSSION

The fact that muscadines as a commercial crop have not heretofore been of much economic importance is probably the principal reason they have received so little attention from plant pathologists. From a cursory examination of numerous plantings in Georgia, it is evident that vineyard losses are considerable in certain seasons as regards both foliage diseases and berry rots. However, since the crop has not been properly evaluated in past years, no monetary comparison can be made between the disease-induced losses of bunch grapes and muscadines.

Although the results of the present investigation do not justify a comprehensive discussion of muscadine diseases, it is not premature to add that muscadines are subject to attack by several diseases other than angular leaf spot. The fact that bunch grape and muscadine culture are coextensive in the Southeast probably in a great measure accounts for the assumption that both are subject to the same diseases. Results of investigations, not yet complete, on other diseases of muscadines indicate an interesting parallel between those of muscadines and bunch grapes. For the most part, however, the pathogens appear to be specifically distinct.

Muscadines are strictly dioecious, and this fact, combined with the observation that individual male vines show variable evidence of infection by the several prevalent diseases, is suggestive that care in the selection of breeding stock may yield resistant varieties of superior quality. In this connection the writer has had occasion to check back on the original male parent used in the production of several of the better quality varieties introduced by this Station and found this parent extremely susceptible to both angular leaf spot and muscadine black rot. Other male vines, apparently more resistant might have yielded more resistant progenies had disease resistance been considered during the breeding program.

SUMMARY

The symptomatology and etiology of a foliage disease, herein designated angular leaf spot of muscadines, has been studied over a period of two seasons. The lesions first appear as small chlorotic areas on the upper leaf

surface. Rapid development results in the production of angular lesions, visible on both leaf surfaces, surrounded by halos on the upper surface. The halos tend to disappear with age. The disease is probably coextensive with muscadine culture in the Southeast.

The pathogen, heretofore known as *Cercospora brachypus* Ell. and Ev., was observed producing spermogonia and perithecia in addition to conidia. Apparently, the perfect stage has not been previously described and is herein designated **Mycosphaerella angulata** n. sp.

Results from a single season's work indicate that angular leaf spot may be controlled by properly timed applications of a 4–5–50 Bordeaux spray. It also is suggested that strict vineyard sanitation may prove an important supplement to spraying.

On the basis of observation alone, it is suggested that a judicious selection of resistant breeding stock may be expected to produce more resistant progenies of superior-quality muscadines.

GEORGIA EXPERIMENT STATION
EXPERIMENT, GEORGIA.

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WIND DISSEMINATION OF ANGULAR LEAF SPOT OF COTTON

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Wind-blown dust as a disseminating agent of the angular-leaf-spot bacterium (*Phytomonas malvacera* (E. F. Sm.) Com. S.A.B.) probably has received less attention than wind-blown rain[1] because dust seldom plays an important role in the dispersal of the pathogen. Dust dispersal of the organism, however, has previously been reported from Oklahoma.[2] In Arizona, where these studies were made, the situation was perhaps more definite, since attendant circumstances made the prevailing conditions fairly comparable to a planned scientific experiment.

An experiment planned to demonstrate the natural spread of the angular-leaf-spot germ by wind-blown dust would necessitate, besides the moving dust, (a) a large plot or field of infected cotton as a source of inoculum, and (b) a noninfested field to which the bacterium could be carried. Such an experiment might involve also the wounding of the healthy plants at the proper time to make sure of infection, in case the bacterium reached the host. Since flowing irrigation water, in contact with infected plants, carries the germ of angular leaf spot and black arm, and may subsequently spread the disease to healthy plants that it laves, fields of infected and healthy plants should be individually irrigated with water from separate clean sources. In location, fields of healthy plants on the one hand and diseased ones on the other should bear the proper relation with respect to the prevailing wind, so that the dust will be carried from the former to the latter and not contrariwise. In order properly to evaluate the results, an isolated control field planted with seed of the same variety of cotton would undoubtedly be prepared. The specified conditions are not usually encountered in commercial cotton growing; indeed, if the conditions were planned by a careful investigator, the experiment would probably fail because nature cannot be depended upon to provide the dust storm, even in Arizona. Yet, exactly the described conditions occurred in southern Arizona in 1940, including a local hailstorm that conveniently wounded the healthy cotton plants that were to receive the inoculum. The area subjected to dust and hail is here designated as District 1; that containing the control plantings, as District 2 (Fig. 1).

GENERAL CONDITIONS

Climatic conditions in both districts were essentially similar, since the two districts are less than 15 miles apart in a direct line. The prevailing winds of summer are from the east and southeast. Precipitation is low and irrigation is largely depended upon for water supply. Showers are some-

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[1] Faulwetter, R. C. Dissemination of the angular leaf spot of cotton. Jour. Agr. Res. [U. S.] 8: 457–475. 1917.

[2] Rolfs, F. M. Dissemination of the bacterial leaf spot organism. Phytopath. 25: 971. 1935.

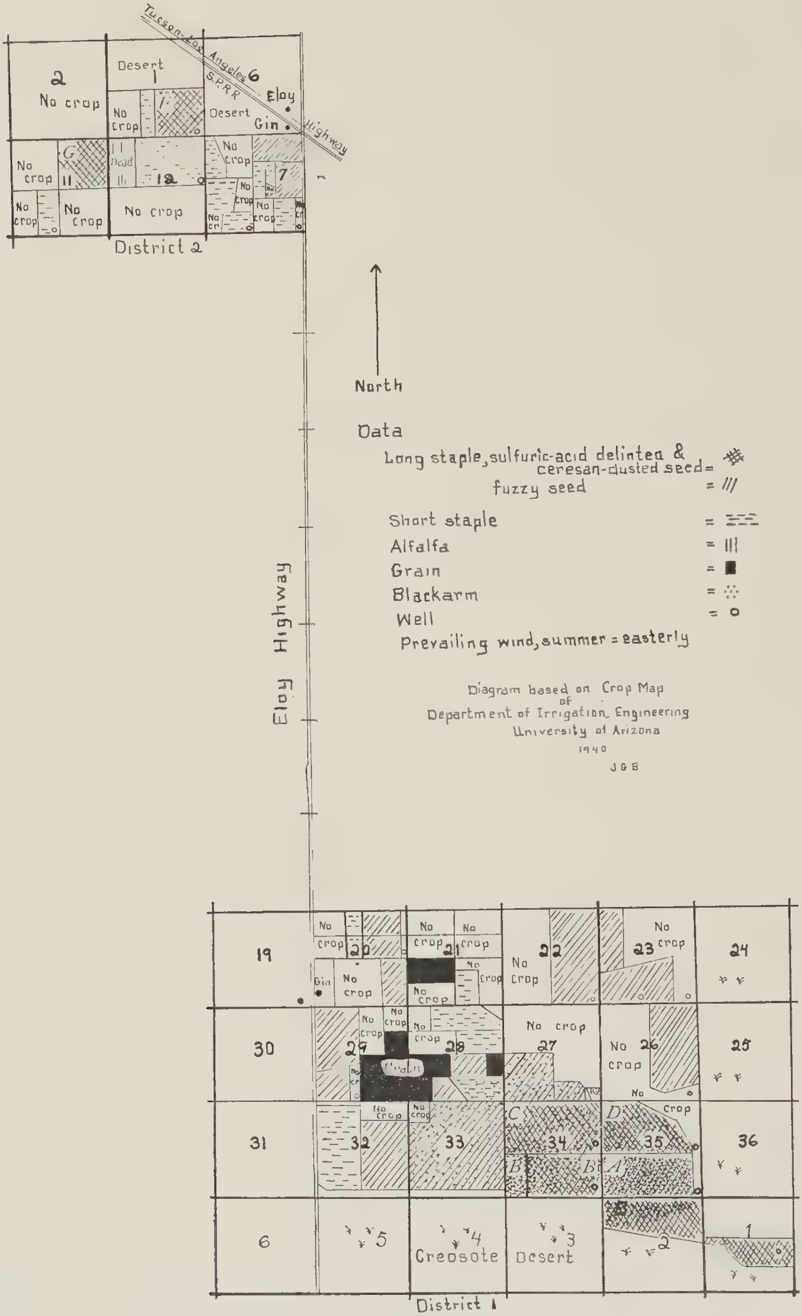
Tucson-Los Angeles
S.P.R.R.
Highway
Eloy
Gin
Desert
No crop
District 2
District 1
North
Data
Long staple, sulfuric-acid delinted & ceresan-dusted seed = ▩
fuzzy seed = ///
Short staple = ☰
Alfalfa = |||
Grain = ■
Blackarm = ⁘
Well = o
Prevailing wind, summer = easterly
Diagram based on Crop Map of Department of Irrigation Engineering University of Arizona 1940
J G B
Eloy Highway
Grain
Creosote
Desert
A
B
C
D
G

FIG. 1. Map showing location of cotton fields studies in Arizona.

times local, reaching only a few hundred acres; this is particularly true of hail storms. The soil has a fine texture and, when thoroughly dry, forms a powdery dust.

The districts were without serious pathological complications, although alternariosis had caused approximately as much damage as angular leaf spot and black arm, in District 1, and considerable damage in District 2. Other diseases of cotton, including verticillium wilt, root knot, and Texas root rot were absent, excepting a very small area of root rot in one field (Field D).

FIELDS STUDIED

The fields that supplied the inoculum consisted of approximately 320 acres and 80 acres, respectively. They were planted with nontreated seed of the SXP variety and irrigated only with water from wells in the same field. The larger field (Fig. 1, A, S½ of Section 35) was separated from adjacent cotton fields on 3 sides by dry, usually dusty, roadways, 60 to 100 feet wide, and on the fourth side it was bordered by desert. The smaller field (Fig. 1, B′, W½ of the SW¼ of Section 34) likewise had broad, dry roadways on 3 sides, but was not separated from cotton on the east. Of the fields of cotton that received the inoculum (Fig. 1, D, approximately the N½ of Section 35; Fig. 1, C, the N½ of Section 34; Fig. 1, B, the eastern part of the S½ of Section 34; and Fig. 1, E, part of the N½ of Section 2), all were isolated one from another by broad, dry roadways, excepting Field B adjoining on the west, without barrier, Field B′. For convenience the fields referred to in this paragraph are collectively designated as District 1 (Fig. 1).

Cotton fields regarded as controls for the purpose of comparison were located in District 2 (Fig. 1), approximately 11 miles north and 4 west of the district mentioned in the preceding paragraph. They were the SE¼ of Section 1 (Fig. 1, F) and the NE¼ of Section 11 (Fig. 1, G) west of Eloy. Field F was separated by a roadway of standard width from desert on the east and from fields of short-staple (Acala) cotton on the southeast and south; on the west a field of Acala cotton adjoined the field of SXP variety without a barrier. To the north lay the desert. Field G adjoined a roadway on the east and was completely isolated from other cotton fields, excepting the southwest corner, which lay opposite a small field of Acala cotton. These fields were planted with sulphuric acid-delinted, Ceresan-dusted cotton seed from the same lot used in the south district.

All fields directly concerned in these studies were planted with the SXP variety of long-staple cotton. Delinted seed was treated by machine with concentrated sulphuric acid, washed, dried, and dusted with 2 per cent Ceresan. In the process, all light-weight seeds were separated from the heavy seeds. "Fuzzy seed" (a term here applied to the seed as it comes from the gin) was not treated. The planting rate for the delinted seed was 9 lb. per acre and for the fuzzy seed 25 lb. per acre. All fields were watered from individual wells. The fields in the southern district (Fig. 1, Sections 34 and 35) had never before borne a crop, having been broken from the desert last year. The larger, A, of the two fields from untreated seed was the sole source of inoculum east of the fields that became infested later.

Only two species of plants other than cultivated cotton[3, 4] are known to be susceptible to the angular-leaf-spot bacterium, and neither species is found in this district.

Black-arm Infested Fields.—The study of the fields was occasioned by the appearance of the black-arm phase of angular leaf spot in cotton grown from acid-delinted and Ceresan-dusted seed, a condition so unusual as to excite comment. The angular-leaf-spot phase was practically absent. Cotton fields from delinted seed, in the northwestern part of the district (District 1, Fig. 1), were free from angular leaf spot when they were examined by the writer in the spring, but an occasional infected plant was found in fields planted with fuzzy seed. The first report of the presence of the disease in fields from treated seed came to the writer early in September, although farmers stated that black arm appeared in August. Examination of the fields verified the presence of the disease in destructive form. The following picture of the situation will be clearly understood by referring to the map (Fig. 1).

Plants in fields B and B′ were most affected by black arm. The stand was almost perfect, yet there were not enough bolls to justify harvesting. Individual plants in Field B showed black-arm lesions mostly extending from the middle, or a little below, to the top; nearly all the lesions were around wounds (Fig. 2, A, B).

The uniform distribution of black arm throughout Field B, as well as the location of the lesions on the infected stems, could not be reconciled with the suggestion of dissemination by irrigation water. In that case, infected plants are more numerous near the irrigation ditch and the stages of black arm are more advanced as compared with plants farther removed from the source of infection. Furthermore, resulting lesions are located at the base of the stem, since infection from irrigation water is limited to the seedling stage.

Field B′, planted with fuzzy seed and adjoining Field B without a barrier, had even fewer bolls than Field B. Individual plants mostly showed lesions from the middle part to the top, but an occasional plant (Fig. 2, C, D) had black-arm lesions at the base.

Field A, planted with fuzzy seed, had a stand like that in Fields B′ and B, but matured very little cotton. Black-arm scars were found on the bases of some plants as well as around wounds on the stems. The wounded plants were more numerous in the western part of the field.

Field D, planted with acid-delinted and Ceresan-dusted seed, yielded more cotton than the fields previously described. Black-arm lesions were located mainly from the middle, or somewhat below, to the top of the plants. Like the other affected fields, Field D showed loss of bolls caused by the angular-leaf-spot bacterium (Fig. 3, D) and by Alternaria. Wounded plants were most numerous in the southern and western parts of the field.

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[3] Brown, J. G., and Frederick Gibson. A new host for *Bacterium malvacearum.* Phytopath. 13: 455–457. 1923.

[4] Palm, B. T. Eriodendron as host of *Bacterium malvacearum.* Phytopath. 22: 867–868. 1932.

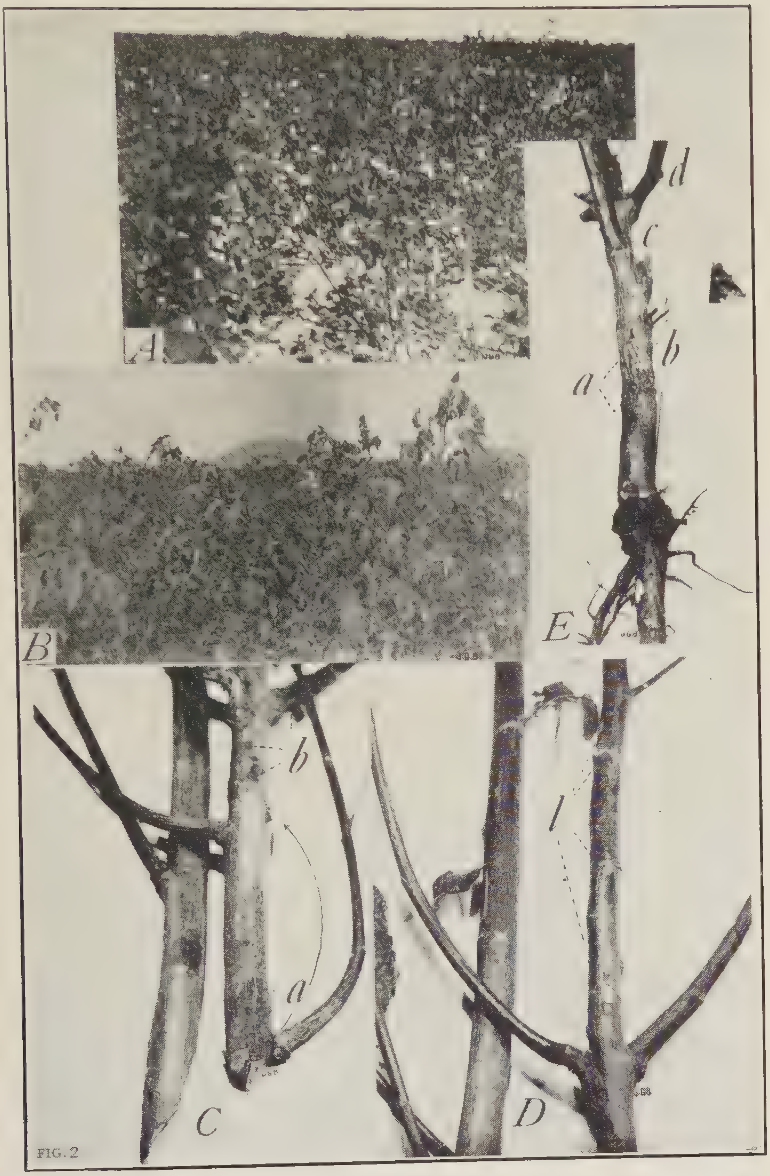

FIG. 2. A. Field (Fig. 1, District 2, F) of S×P cotton taken after first picking. The field was planted with sulphuric acid-delinted and Ceresan-dusted seed, from the same lot used in Fields B, C, and D in District 2. There was no black arm in this field. B. Field of S×P cotton (Fig. 1, District 1, D) grown from sulphuric acid-delinted and Ceresan-dusted seed. Black arm appeared late in this field and was worse on the south side adjacent to a field (Fig. 1, A) grown from untreated seed. C. Lower parts of stems of cotton plants, left, from clean Field F; right, from black-arm-infested Field B′. The dark spot near the base of the plant on left is a small leaf and its shadow. a. Inactive black-arm lesions from seedling stage. b. Black-arm lesions higher on stem; arrow points to former attachment of black-arm-affected branch. D. Upper parts of cotton stems illustrated in C. Stem on left, grown from treated seed, is clean. l. Extensive black-arm lesion on stem of plant from untreated seed; the dead bark is separated from the sound bark below and along the side by a deep crack. The top of the plant was killed by black arm. E. Cotton plant grown from untreated seed (Field B′). The plant was stunted and apparently had been affected with black arm since the seedling stage. a. Inactive basal stem lesion from which the angular leaf spot bacterium was isolated. b, c. Infection spots linking basal lesion with black-arm-blighted top, d.

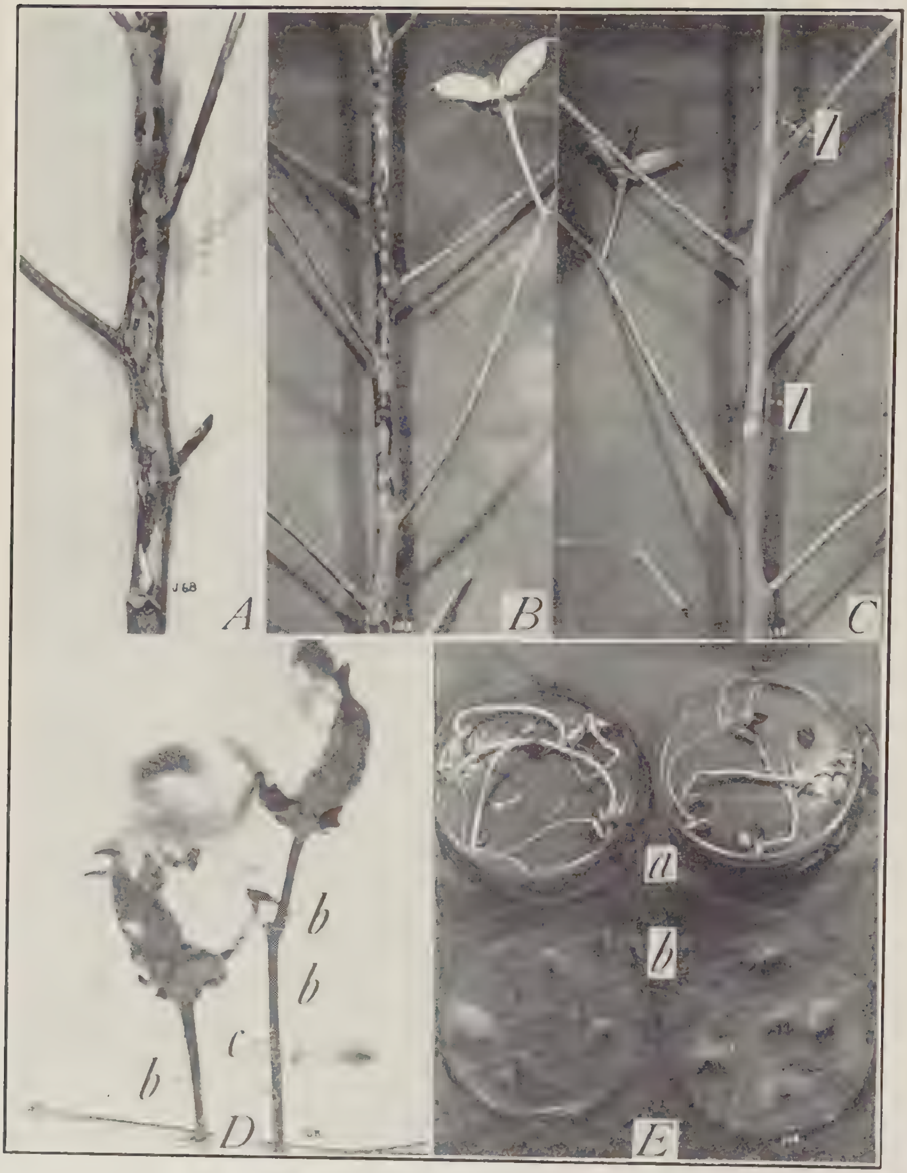

FIG. 3. Piece of cotton stem from Field B (Fig. 1) showing hail wounds, each wound more or less surrounded by a black-arm lesion. B. East side; C, west side of part of the main stem of a cotton plant from Field B (Fig. 1); l, margin of lesion mainly on opposite side of stem. This plant was typical for the hail-injured, black-arm-affected plants in the fields from treated seed. D. Cotton bolls and pedicels with glistening black arm lesions, b, and thin, pale, dried flecks of bacterial ooze, c. E. Cultures of cottonseeds from the lot left after planting the fields studied: a. Sulphuric acid-delinted and Ceresan-dusted seeds. b. Untreated, fuzzy seeds. Cultures of the treated seed usually were clean; cultures of the untreated seeds gave mold and other fungi, also colonies of bacteria, including *Phytomonas malvacera.*

Field C, planted with sulphuric-acid-delinted, Ceresan-dusted seed, showed plants with less black arm than those in Fields B and A and also produced more cotton. Black-arm lesions were mainly from the middle region of the plants to the top; they were associated with wounds.

In Field E, planted with acid-delinted, Ceresan-dusted seed, the plants were affected by black arm only on the north and west sides of the field, in a triangular area, with the apex in the northeast corner and the base along the west side. In this area plants had black-arm lesions that extended from the middle region of the stem toward the top and were associated with wounds.

The presence of wounds on the cotton plants is explained by the occurrence of a local hailstorm on August 13. Serious wounding of cotton plants resulted in Fields A and E and westward at least to Section 32, and northward over District 1. On August 20, a windstorm, violent enough to tear leaves from plants and hurl them over a 100-foot wide roadway, swept the same area. Both storms very evidently came from the east, the generally prevalent direction of summer storms in southern Arizona. Local residents state that unlatched east doors of their homes were blown inward; hail wounds resulting from the first storm were mostly on the east sides of the main cotton stems. (Fig. 3, B, C).

The "Control" Fields.—For comparison with the cotton crop in District 1, two fields may be used that were planted with sulphuric acid-delinted, Ceresan-dusted seed from the same lot used in Fields B, C, and D. That these fields (Fig. 1, F, G) were partly isolated from others of long-staple cotton, may be seen at a glance, although both were adjacent to plantings of the Acala variety which is less susceptible to black arm. No black arm was found in Fields F and G. Four bolls affected with rot induced by the bacterium of angular leaf spot were collected in Field F, of which 3 showed insect punctures in the decayed spots; 5 similarly infected bolls were found in Field G. The bolls were mostly "top crop" and the infection therefore late. Many bolls had been killed by an Alternaria.

THE SEED

The culturing of cottonseed from the lots used in planting the fields was carried out. Plenty of the acid-delinted seed was available, but the only fuzzy seed left after planting was a small quantity removed from the planter and dumped on the ground. Representative samples of the cultures of the delinted seeds (Fig. 3, E, a) and of the fuzzy seeds (Fig. 3, E, b) are illustrated. The acid-delinted and Ceresan-dusted seeds usually gave no organisms whatever, although some seeds that were cultured were obtained from the dust on the ground where they had spilled from torn sacks; in no case did cultures of the delinted, dusted seeds give a yellow bacterium. The fuzzy seeds, as usual, gave a number of fungi and a pale yellow bacterium that became a deeper yellow as it grew older. This was the bacterium of angular leaf spot and black arm, *Phytomonas malvacera*, as subsequently proved by further planting, inoculations, and reisolation.

DISCUSSION

From the evidence presented by plants in the fields described above, it is clear that primary (seed-derived) infection with the angular-leaf-spot and black-arm germ existed in Fields A and B′; likewise clear that such infection did not occur in Fields B, C, D, and E. Supporting evidence are the healed black-arm lesions present on the bases of plants in Fields A and B′; also, lesions in the fields planted with acid-delinted seed and located in and above the middle region of the plants, a condition possible only in late infection.

It is quite probable that infected leaves fell to the ground and became broken and mixed with the dust in the fields first infested. The hailstorm of August 13, which severely wounded the growing plants in all fields mentioned, was followed by the inoculum-laden dust storm of August 20. The most abundant inoculum thus transported reached Field B, directly west of Field A (fuzzy, untreated seed); also, some inoculum was carried into the north side of Field E in an area widening westward; some into Field D, the heavier dose reaching the south side, and some into Field C, likewise most severely infested on the south side. Field B′ probably contributed little if any inoculum to Field B, but cotton growing farther west, in the direction of the moving dust, may have received inoculum from Field B′, since black arm was present in Section 33 (Fig. 1).

The effectiveness of hail in increasing infection with the angular-leaf-spot bacterium is very well illustrated in these studies (Fig. 2, A, B). Properly timed with moving, inoculum-charged dust, a hailstorm apparently is important in the spread of angular leaf spot and black arm. In the fields here discussed, the infection with black arm was very uniform in development over an area 2 miles long, east to west (Fig. 1, south parts of Sections 35 and 34), whereas Faulwetter[5] observed the spread of inoculum by wind-blown rain over a much smaller area. Even allowing for the comparatively limited source of inoculum in Faulwetter's studies, wind-blown dust must be regarded as a more effective carrier. Comparison of the studies in Arizona with Rolf's[6] observations serves to emphasize the destructiveness of hail in combination with moving dust. Rolf observed that "a single whirlwind scattered the infected dry leaf material over 100 acres in 20 minutes." In the Arizona storm preceded by hail, the infected material started disease over more than 1,000 acres.

The almost total absence of the angular-leaf-spot phase of disease following the hail and dust storms is significant. Although the leaves of cotton plants in District 1 must have been wounded by hail and contacted by the inoculum-laden dust, almost no infection resulted. Presumably, the surface of the leaves exposed to the air dried before the bacterium of angular leaf spot could start disease, whereas, the juicier wounded stems afforded sufficient moisture long enough to permit the growth and multiplication of the organism. Rapid drying of the surface of susceptible plant parts may

[5] See footnote 1.

[6] See footnote 2.

explain the comparatively rare dissemination of angular leaf spot by windblown dust.

SUMMARY

A field of 240 acres of the black-arm-susceptible SXP cotton plants grown from sulphuric-acid-delinted and Ceresan-dusted seed was extensively and uniformly infested and adjacent fields less affected, in September, with the black arm phase of angular leaf spot. The field with most severely infected plants lay directly west of a half-section of land planted with nontreated or fuzzy seed, and it adjoined on the west, without barrier, an 80-acre field also planted with untreated seed.

A field of cotton from delinted and dusted seed that lay north of the half-section from nontreated seed contained more diseased plants on the side adjacent to the latter field. The ranch south of the half-section from fuzzy seed had black arm only in plants on the side of the ranch next to the half section.

Although the stand of cotton in all the fields was good, evidence existed that black arm was present in the fields from fuzzy seed and not present in the fields from delinted and dusted seed in the seedling stage of the crop. This evidence agrees with the observations made in the spring a few miles farther north and in the same district.

No source of black-arm inoculum existed other than the cultivated cotton. The inoculum could not have been carried into the fields from treated seed by irrigation water, for the fields were individually irrigated from deep wells and separated one from another by dry roadways; furthermore, black arm was quite uniformly distributed over the fields from treated seed and fairly uniformly developed in the plants from treated seed, rather than more extensive and in later stages in plants near irrigation ditches and in earlier stages in plants farther removed from the ditches.

Cultures of acid-delinted and Ceresan-dusted seed from the same lot planted in the fields from treated seed usually gave neither bacteria nor fungi, and in no case did they give a yellow bacterium. Cultures of the fuzzy seed from the same lot planted in the fields from untreated seed gave a luxuriant growth of fungi and also yellow colonies of bacteria that were proved to be the angular-leaf-spot (black-arm) germ.

The cotton fields were swept by a hailstorm on August 13 and by a dust storm one week later. The general direction of the storms was east to west. Prior to the storms no destructive action of black arm had been noticed. The hailstorm wounded the cotton plants and the dust storm disseminated the inoculum of black arm. By September, infection with the bacterium of black arm was uniform throughout the fields from treated and untreated seed.

Cotton fields here used as checks or controls against the black-arm infested fields were planted approximately 12 miles distant from the latter, by the same farmers, with sulphuric acid-delinted and Ceresan-dusted seed from the same lot used in the infested fields, in like soil, under similar conditions excepting hail injury and subjection to violent dust storm. The control fields were isolated from other fields of the black-arm-susceptible, long staple (SXP) cotton. No black arm was found in the control fields.

Conclusions were: (a) that the black-arm inoculum was carried in dust from the half section of land bearing infected cotton plants reared from fuzzy seed, that the heaviest dose of inoculum reached the cotton field on the west, but that the inoculum-bearing dust spread laterally westward in a somewhat fan-shaped belt to reach bordering fields on the north and on the south while inoculum from the smaller field reared from fuzzy seed was also carried westward at the same time; and (b) that since the area of cotton directly affected included more than two sections of land, wind-blown dust following a hailstorm was most efficient in disseminating the bacterium of angular leaf spot.

Pursuance of these studies was facilitated by the absence of all seed-borne diseases from the crop, except black arm, and the almost absolute freedom of the fields from diseases such as verticillium wilt, Texas root rot and root knot that are caused by soil-dwelling parasites. On the other hand, part of the damage attributed to black arm by farmers was caused by the air-distributed fungus, *Alternaria* sp.

As an addendum for any cotton grower into whose hands, perchance, this paper may fall, it is pointed out that, although the combination of meteorological events that contributed to the heavy loss (estimated to total $60,000 for the two farmers concerned) is not a common occurrence, mixed planting of treated and untreated cotton seed is unwise.

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TUCSON, ARIZONA.

A Xylaria Pathogenic to Ginkgo biloba L. Seeds.[1]—In the course of a pathological survey of the Morris Arboretum, Philadelphia, Pennsylvania, in the summer of 1938, a species of *Xylaria,* tentatively identified as *X. longeana* Rhem, was isolated from dying and dead branches of a staminate specimen of *Ginkgo biloba* L. Experimental inoculations of seeds, seedlings, and branches of mature trees were studied to determine the pathogenicity of the fungus. The results with seedlings and mature trees are as yet incomplete.

Seed was collected in the fall of 1938 and of 1939. The outer fleshy layer was removed by washing and the seed then stored in a cardboard carton in a refrigerator at 40° F. The seeds were planted the April following collection, one-half inch deep in native clay loam in twelve-inch pots, and kept under ordinary greenhouse conditions. The inoculation material consisted of the fungus, which was growing in steam-sterilized wheat. This was incorporated into the soil at the time the seeds were planted. Since no significant difference was noted in the results observed with sterilized and unsterilized soils in the first series, only the latter were used in subsequent experiments.

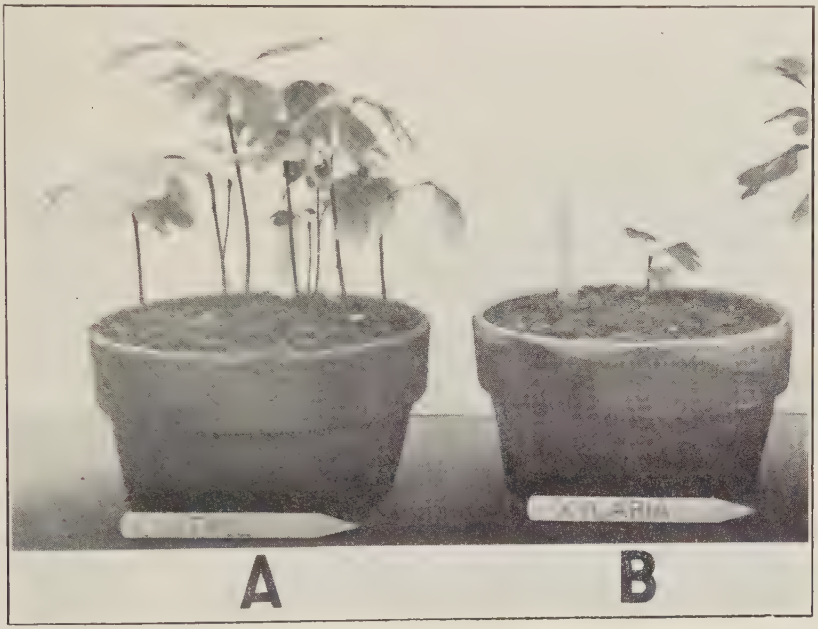

FIG. 1. Seedlings 5 months after planting. A. Control. B. Inoculated. ×$\frac{1}{7}$.

Good emergence occurred in the control pots over a period of ten weeks, while the seedlings in the inoculated pots were badly stunted (Fig. 1). In the inoculated pots the seeds that did not germinate and the seedlings that

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[1] Acknowledgement is made to Dr. Harlan H. York for helpful suggestions in the course of this work.

TABLE 1. *Percentage emergence of seedlings of Gingko biloba in sterilized and nonsterilized soil certain pots of which were inoculated and others left as controls*

Year	Soil	Culture	Emerged
			Per cent
1939	Nonsterile	Controls	32.0
	Sterile	Controls	36.0
	Nonsterile	Inoculated	2.0
	Sterile	Inoculated	2.0
1940	Nonsterile	Controls	55.4
	Nonsterile	Inoculated	2.5

failed to emerge were covered with thick, black mycelial masses resembling stromata (Fig. 2, D). From such seeds fruiting bodies of the *Xylaria* had pushed up above the surface of the soil. Removal of these masses of mycelium from the stony layer of the seed revealed black zone lines[2] and spots (Fig. 2, E), which were extended through the stony layer into the papery layer of the testa (Fig. 2, F and G). Seeds that failed to germinate or were

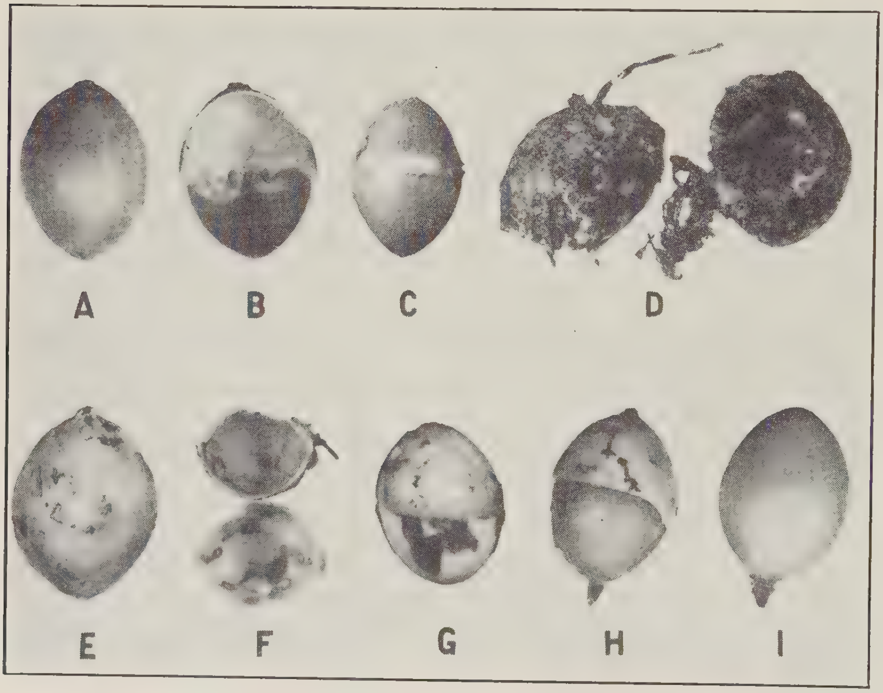

FIG. 2. A. Normal seed with stony layer unbroken. B. Normal seed showing inside appearance of stony layer. C. Normal seed with stony layer removed. D. Seeds from inoculated soil; black mycelium and fruiting body of *Xylaria* on seed. E. Mycelium scraped from seeds in D, showing black zone lines on outer surface of stony layer. F. Seed from D, showing inside appearance of stony layer. G. Seed from D with stony layer removed. H and I. Seeds from inoculated pots, killed soon after germination. All ×1.

[2] Hilborn, M. T. The Anatomy of a black zone caused by *Xylaria polymorpha*. Phytopath. **27**: 1177. 1937.

killed in the preemergence stage (Fig. 2, H and I) were cultured and the *Xylaria* reisolated.—SPENCER H. DAVIS, JR., and JOHN B. HARRY, The Morris Arboretum, Department of Botany, University of Pennsylvania, Philadelphia, Pa.

A Virosis-like Injury of Snapdragon Caused by Feeding of the Peach Aphid.—Snapdragon seedlings and cuttings grown for experimental purposes under glass at Los Angeles in the Spring of 1940 commonly showed an injury of the terminal growth, which resembled a virosis. Subsequently, the same injury was observed on *Antirrhinum* plants, growing both under glass and in a protected outdoor location in the San Francisco Bay area, and again in the Spring of 1941 under glass in Los Angeles. Careful examination of the plants showed the presence of no pathogen and only occasional aphids or their cast skins. It appeared that the disease was caused by a virus or resulted from the feeding of the aphids.

The genetical value of some of the plants made desirable their vegetative propagation, but the cessation of growth of diseased shoots would have made this unsuccessful if a virus had been involved. Tests were, therefore, undertaken to determine the cause of the disease.

The injury has been observed occurring naturally on *Antirrhinum majus* L., *A. speciosum* Gray, *A. nuttallianum* Benth., *A. glandulosum* Lindl., *A. virga* Gray, *A. molle* L., and *Linaria dalmatica* Mill. The symptoms were essentially alike on all these species.

The most characteristic feature of the injury was that apical growth was checked and each rosette of leaves, when viewed from above, had a pale yellow to white center with a periphery of green, or of green mottled or spotted with yellow. Stem tips thus injured often became brown and died, but sometimes resumed growth after a time (Fig. 1, C) or gave rise to new lateral branches (Fig. 1, D).

The leaves were greatly reduced in size and frequently were rolled, dorsally curled, and laterally distorted (Fig. 1, A). Sometimes there were yellow or white spots 1 mm. in diameter in the laminae of apical leaves, particularly near the veins (Fig. 1, B). The midrib and principal veins were yellow to white and the pallor extended somewhat to the surrounding tissues, particularly at the leaf bases. Rugosity occurred only in cases in which yellow areas partially or wholly surrounded a green island. Injured leaves have never been observed to recover. Sometimes, particularly in a tetraploid and in a double-flowered diploid variety of *A. majus*, persistent, bright yellow, circular spots up to 5 mm. in diameter developed in the basal leaves.

The injury was found to be due to feeding of the peach aphid (*Myzus persicae* Sulz.[1]) Previously healthy snapdragon seedlings showed typical injury of the leaves on which small numbers of these aphids had fed. New foliage formed on either a continuation of the same axis or its laterals,

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[1] Determination by E. O. Essig.

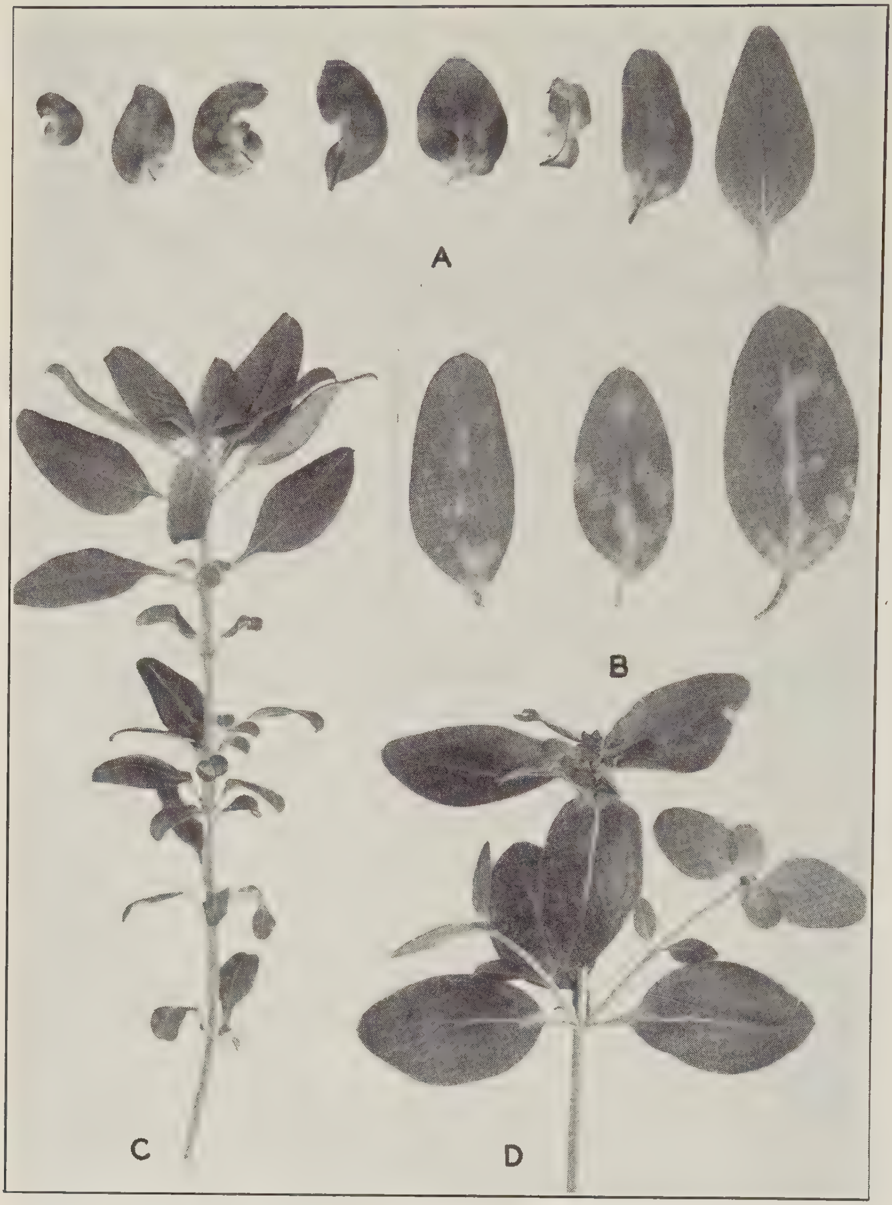

FIG. 1. Virosis-like injury to *Antirrhinum* spp. caused by feeding of *Myzus persicae*. A. Apical leaves of *A. majus*, showing distortion and discoloration; check at right. B. *A. speciosum* leaves with characteristic pallid spots. C. *A. majus* with stunted and distorted lower leaves and normal terminal growth produced after removal of aphids. D. *A. majus* with development of growing point completely checked by aphids and normal new growth arising laterally following removal of aphids.

following the removal of the aphids, was entirely normal under environmental conditions in which infested plants contined to show symptoms (Fig. 1, C). Peach aphids from a non-viruliferous line that had been maintained for many generations on turnip gave symptoms on *Antirrhinum* identical to those caused by insects taken directly from diseased snapdragons. Plants showing the injury outdoors developed healthy new growth when freed of aphids and either taken into a glasshouse or kept outdoors; plants that had not been freed of aphids contined to show symptoms. Plants that had recovered did not show symptoms again in 4 months time. Only leaves on which aphids had fed have showed these symptoms. Plants with several shoots have shown symptoms only on the specific branch on which the insects have fed.

Attempts to transmit a virus, by the carborundum method, from diseased snapdragons to Turkish tobacco, *Nicotiana glutinosa,* spinach, tomato, cucumber, and snapdragon were unsuccessful.

It is concluded that the injury was caused by feeding of the peach aphid, probably resulting from some toxic secretion of the insect. The aphid is so toxic that a few insects can cause a surprising amount of injury, particularly because they collect at the tender growing points. Although the aphid can build up high populations when caged on snapdragon, it has not been seen to do so on unprotected plants. Plants with both aphids and the injury they cause have consistently recovered when planted in the field under southern California conditions.—KENNETH F. BAKER and C. M. TOMPKINS, Division of Plant Pathology, University of California, Berkeley, California.

Technique for Artificially Feeding Scolytus multistriatus and Saperda tridentata Spores of Ceratostomella ulmi and Other Substances.—It is now established that *Ceratostomella ulmi* (Schwarz) Buisman is disseminated chiefly by *Scolytus scolytus* Fab. and *S. multistriatus* Marsh. in Europe and by the latter species in the United States. Grossmann[1] and Betrem[2] determined that spores of *C. ulmi* were carried internally and externally by *S. scolytus.*

Collins *et al*[3] reported the isolation of *Ceratostomella ulmi* from adult *Scolytus multistriatus,* captured while crawling on elm logs in the field. But no record has been found of work to determine whether the fungus is carried internally or eliminated in the feces by *S. multistriatus.*[4] To study this and other relationships between the beetle and fungus, a technique was devised for artificially feeding the beetles under conditions precluding external transfer of the fungus or other substances from the mouthparts to the posterior portion of the body and fecal pellets.

[1] Grosmann, Helene. Beiträge zur Kenntnis der Lebensgemeinschaft zwischen Borkenkäfern und Pilzen. Zeitschr. f. Parasitenk. 3: 57–102. 1931.

[2] Betrem, J. G. De Iepenziekte en de Iepenspintkevers. Tijdschr. over Plantenziekten 35: 273–288. 1929.

[3] Collins, C. W., W. D. Buchanan, R. R. Whitten, and C. H. Hoffmann. Bark beetles and other possible insect vectors of the Dutch elm disease, *Ceratostomella ulmi* (Schwarz) Buisman. Jour. Econ. Ent. 29: 169–176. 1936.

[4] Since the preparation of this manuscript, Parker *et al.* have reported the isolation of *C. ulmi* from the gut of *Scolytus multistriatus.* Parker, K. G., Philip A. Readio, Leon J. Tyler, and Donald L. Collins. Transmission of the Dutch elm disease pathogen by *Scolytus multistriatus* and the development of infection. Phytopath. 31: 657–663. 1941.

Active, recently emerged adults, free of *Ceratostomella ulmi,* were stuck to the ends of tapered matchsticks with cellulose glue. The legs were fastened to the end of the stick, leaving the head and the posterior part of the abdomen free.

A small stand (Fig. 1, A) was constructed from 2 pieces of wood. The matchstick with the attached beetle was stuck in a hole near one end of the base. A glass tube was mounted on the end of another matchstick, inserted in a radio binding post. The stick was adjusted so that the head of the beetle was just inside the end of the tube (Fig 1, B). The tubes were loaded with the various substances to be ingested. Air currents and dust were kept from the apparatus by a celluloid cover. Beetles freely ingested food for as much as 15 days and water and other substances for several days while mounted on the matchsticks. In other experiments predetermined small quantities of spore suspensions were injected directly into the mouths of the beetles with the aid of a micromanipulator and a micro-pipette.

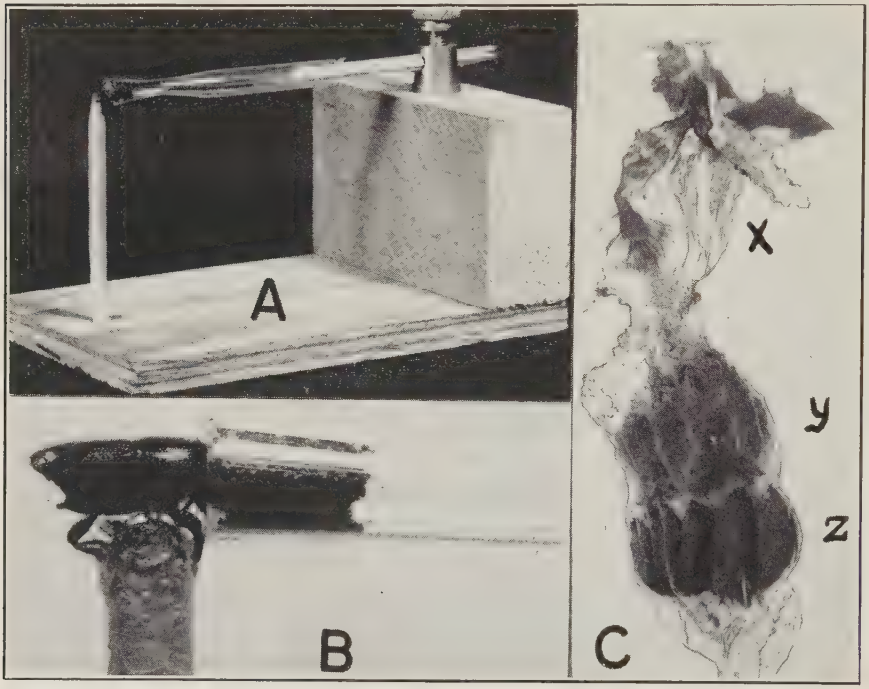

FIG. 1. A. Apparatus for artificial feeding of *Scolytus multistriatus.* × ¾. B. A beetle with legs glued to the end of a stick and head inserted in feeding tube. × 4. C. Foregut of *Scolytus multistriatus* that had fed on spore suspension of *Ceratostomella ulmi* mixed with elm bark: x, oesophagus; y, ingested food in crop; z, proventriculus. × 60.

Coremia of *Ceratostomella ulmi,* spore suspension of that fungus, spore suspension of *C. ulmi* mixed with finely ground elm bark, water, arsenic, and a solution of Neoprontosil were fed to the beetles. Feeding was readily observed under a binocular microscope.

The entire digestive tract was dissected out of several beetles and observed under the microscope.[5] Ground bark, similar to that fed the beetles, was found in all parts of the gut and in fecal pellets voided by the beetles. Beetles usually commenced to discharge such pellets about 1½ hours after they began to feed. Ingested material was observed in the crop (Fig. 1, C, y) of beetles that had fed for short periods. In beetles that had not fed, the entire gut was empty except for the rectal area, which always contained small white pellets of material that was not identified.

Pellets of excrement were obtained from the feeding beetles under conditions precluding their accidental contamination by *Ceratostomella ulmi.* The feet were fastened and could not accidentally transfer contaminated particles from the heads to the posterior parts of the abdomens. The entire posteriors of the beetles were frequently surface-sterilized by brushing with 95 per cent alcohol. The alcohol treatment did not interfere with feeding or prevent elimination of feces.

Adult *Scolytus sulcatus* Le C. and *Saperda tridentata* Oliv. have been fed successfully by the same method.—W. D. Buchanan, Division of Forest Insect Investigations, Bureau of Entomology and Plant Quarantine, and Curtis May, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, Morristown, N. J.

Aecidium gossypii, the Aecial Stage of Puccinia boutelouae.[1]—Connection has been established between *Aecidium gossypii* and a *Puccinia* on *Bouteloua* spp. Observations had indicated that there might be a connection between the uredial and telial stages of the rust on the grasses and the aecia on cotton. The connection was finally established by inoculating cotton with telial material from *Bouteloua aristidoides* and *B. barbata* and then reinoculating these 2 grasses with the resulting aecia on cotton. That cotton is the aecial host for the rust on the *Bouteloua* in nature is indicated by field observations.

In the late summer and fall of 1940 there were heavy epidemics of cotton rust in several widely separated localities of Arizona. There was particularly good opportunity to study the rust at Hidden Valley (Pinal County) and to observe the native vegetation growing on the desert immediately adjacent to cotton fields. In one field especially there was no possibility that the rust had been carried over on some other crop plant, because the field was under cultivation for the first time, having been cleared of desert growth the previous year. The native plants were critically examined in the hope of finding the unknown telial host of the cotton rust fungus; two species of grass, *Bouteloua aristidoides* and *B. barbata*, were found heavily rusted. These observations were confirmed in the Continental area (Pima County) where the same two species of grass were found severely rusted adjacent to an infected field of cotton. The fact that the severity of rust on the grasses decreased with the distance from the cotton planting suggested a relation between the grass rust and that on cotton.

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[5] The writers are indebted to R. T. Webber of the Bureau of Entomology and Plant Quarantine for his assistance in dissecting the gut from a number of beetles.

[1] Cooperative investigations between the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Minnesota Agricultural Experiment Station. Paper No. 1910 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station. The field work was done in the course of the writer's regular duties at the U. S. Dept. of Agriculture field station at Sacaton, Arizona. The greenhouse and laboratory work was done at University Farm, St. Paul, Minn., while the writer was on leave from federal duties and held a research assistantship at the University of Minnesota.

The writer wishes to express his deep gratitude to William Q. Loegering, Agent in the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, stationed at University Farm, St. Paul, Minn., for cooperation in the laboratory and greenhouse work.

Fresh uredial and telial material was collected in September, 1940, and tentatively identified as *Puccinia boutelouae* (Jennings) Holw. This material was stored in a refrigerator; in March, 1941, telia that had overwintered on the desert were collected also. Both collections were used in inoculation studies at the University of Minnesota during April and May, 1941. Several species of *Bouteloua* (*B. aristidoides, B. barbata, B. curtipendula,* and *B. gracilis*) became infected when inoculated with the uredia collected in Arizona the previous fall. Cotton seedlings of the Acala variety, about 2 weeks old, were exposed separately to germinating telia from the fall and spring collections. The seedlings were placed in an incubator and telia that had been presoaked in water were spread on a wire screen above them. After 4 days the first series of seedlings was removed and another series placed under the telia for an additional 4 days. Relatively few pycnia appeared on the plants in the first series, but they were numerous in the second series, indicating that the telia had begun to germinate after approximately 4 days, and had thus caused a much heavier shower of sporidia to fall on the second series of plants.

The formation of aecial cups around the pycnial clusters was noticed about a week after the appearance of the pycnia. As soon as the first aecial cups had matured, plants of *Bouteloua* spp. were inoculated with the aeciospores; 8 days later uredia appeared. The characters of urediospores formed as a result of inoculating *Bouteloua* with aecial material were identical with those of urediospores on the grasses rusted in nature and with those of uredia formed on grasses inoculated with the fall collection of uredial material.

Aecidium gossypii, therefore, evidently is one stage of *Puccinia boutelouae*, as the telial material that caused the formation of aecia on cotton agreed in all respects with the description for this species. Furthermore, the uredial stage as found in nature and that resulting from inoculation of the grasses with the above-mentioned aecia on cotton also conformed in all respects to the description of the species. There seems no question, therefore, that the rust causing infection on cotton is *P. boutelouae.*

There is some question as to whether *Puccinia boutelouae* is a synonym of *P. vexans*, which also occurs on *Bouteloua*. The 2 species are quite similar morphologically, the most pronounced difference between them, according to

Arthur,[2] being the length of the teliospore pedicels. Nevertheless, the writer examined herbarium specimens labeled *P. vexans*, and they agreed with the description of *P. boutelouae*. It may be that there is only one rust or that *P. vexans* and *P. boutelouae* should be considered races of the same species. If it is concluded that there is a single species, the name *P. vexans* would have priority. This entire question, however, must await further observations, experiments, and examination of herbarium material.—JOHN T. PRESLEY, U. S. Field Station, Sacaton, Arizona.

Transmission of Chlorotic Streak of Sugar Cane by the Leaf Hopper Draeculacephala portola.[1]—The cause and the means of natural transmission of chlorotic streak of sugar cane have remained unknown since the disease was first differentiated by Wilbrink in Java in 1928. Most workers who have studied the disease have noted that the nature of spread in the field indicated possible transmission by an insect vector, although proof has been lacking.

Experiments on insect transmission of the disease were conducted in Louisiana by the writers during 1941 in an insect-proof greenhouse. Cages 3 ft. long, 3.5 ft. tall, and 2 ft. wide, with tight wooden bottoms, and covered with 40-mesh copper-wire cloth and very finely woven cotton cloth, were used on ant-proof benches. Into each cage were placed from 10 to 15 insect-free chlorotic-streak-diseased plants of the variety C.P. 29/320 growing in sterilized 4-in. clay pots in soil that had been subjected to flowing steam for 3 to 4 hours, and an equal number of insect-free healthy plants growing in steamed soil from cuttings treated with hot water at 52° C. for 20 minutes, which treatment eliminates the disease. In this note, the term "healthy" is used to refer to plants grown from cuttings so treated.

An average of 5 *Draeculacephala portola* per healthy plant was introduced into each cage and allowed to feed for from 7 to 14 days. The healthy plants were then removed from the cages in a closed room separated from the greenhouse and placed in other cages, where they were fumigated twice at intervals of 7 to 10 days before being returned to the greenhouse. In some instances the diseased plants were removed prior to introducing the healthy ones into cages with leaf hoppers that had been feeding for 2 to 4 weeks on diseased plants. The entire greenhouse was fumigated at intervals of 7 to 10 days.

A total of 490 healthy plants was exposed to the leaf hoppers in this manner, beginning in March, 1941, and continuing to late May. By September 1, 25 of these had developed typical leaf symptoms of chlorotic streak. Twenty of these were from cages containing both diseased and healthy plants, and 5 were from those in which the healthy plants had been exposed to the leaf hoppers following removal of the diseased plants.

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[2] Arthur, Joseph Charles. Manual of the rusts in United States and Canada. Purdue Research Foundation, Lafayette, Indiana, 1934. pp. 172–173.

[1] Although *Draeculacephala portola* Ball has previously been misidentified as *D. mollipes* (Say) feeding on sugar cane in the Gulf States, true *mollipes* appears to occur primarily in the northeastern United States and is not known as a pest of sugar cane in Florida or Louisiana.

As controls, 60 healthy plants were caged with diseased plants, but without leaf hoppers, for the same period of time as those with the insects; between 300 and 400 healthy plants were grown in a bench adjacent to diseased ones in the greenhouse in which the transmission experiments were conducted; and 20 healthy plants were grown for 9 months in a bench of soil in the greenhouse, set alternately at 8-inch intervals with diseased ones, where their roots and leaves were in intimate contact. None of these has shown symptoms of chlorotic streak. In addition, 20 healthy plants have been grown in an insect-proof greenhouse for 2 years in contact with chlorotic-streak-diseased plants and have remained apparently healthy.

While these experiments are of a preliminary nature, they offer proof of transmission of this disease by *Draeculacephala portola*. This leaf hopper is usually more abundant in Louisiana than the total of all other sucking insects found on sugar cane, excluding mealybugs. The question of whether this leaf hopper or close relatives coexist with the disease is of interest. The genus *Draeculacephala* has been reported in Hawaii, Puerto Rico, Colombia, and Louisana, where chlorotic streak apparently spreads under natural conditions, but not in Java, where the disease was first described. Further experiments with this and other sugar-cane insects are in progress.—E. V. ABBOTT, Bureau of Plant Industry, and J. W. INGRAM, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Houma, Louisiana.

Susceptibility of Lee × Victoria Oat Selections to Loose Smut.—Since its introduction into the United States in 1927, the Victoria oat has been used extensively in crosses with other varieties for the development of disease-resistant strains. Several selections from these crosses have been named and distributed. Those from Victoria × Richland and Lee × Victoria have been especially promising for particular regions.

Victoria, with the exception noted below, has been highly resistant to smut. It was inoculated with many collections of loose (*Ustilago avenae* (Pers.) Jens.) and covered (*U. levis* (Kell. and Sw.) Magn.) smuts in numerous field and greenhouse experiments, and has been fully resistant to 29 races of loose smut and 14 of covered smut. It also is markedly resistant to almost all races of crown rust (*Puccinia coronata avenae*).

A specimen of loose smut (Collection 50) from Fulghum oats, collected by the junior writer at Stillwater, Oklahoma, in 1934, was sent to the Brooklyn Botanic Garden, where it was found to produce smut in Victoria and in certain hybrid strains derived from that variety.

Data on the infection of 22 selections from a Lee × Victoria cross are given in table 1.

The Lee × Victoria cross, from which these 22 promising selections were derived, was made by the junior writer in 1931. The selections listed were

TABLE 1.—*Reaction of Lee and Victoria oat varieties and selections from crosses between them to a newly discovered race of loose smut at the Brooklyn Botanic Garden, Brooklyn, N. Y., in 1941*

C. I. No.	Selection No.	Variety	Plants grown	Plants infected	
			Number	*Number*	*Per cent*
		Parental varieties			
2042	....	Lee	27	25	92.6
2401	....	Victoria	16	7	43.8
		Selections			
3379	P1–7–3–1	Lega	17	2	11.8
3384	P1–7–4–1	Levic	19	9	47.4
3392	P1–20–4–1	Letoria	24	12	50.0
3393	P5–9–3–2	Lenoir	19	11	57.9
3969	P32–4–1–2	....	20	2	10.0
3400	P34–1–2–1	....	15	1	6.6
3402	P34–1–2–3	....	14	1	7.1
3404	P34–1–3–1	Lelina	21	10	47.6
3405	P34–1–3–2	....	17	7	41.2
3406	P34–9–1–1	....	25	17	68.0
3609	Coker 38–57	....	23	11	47.8
3855	Coker 40–5	Stanton	10	2	20.0
3936	Coker 40–6	....	28	6	21.4
3944	Coker 40–33	....	23	9	39.1
3945	Coker 40–34	....	18	10	55.6
3946	Coker 40–35	....	24	9	37.5
3947	Coker 40–38	....	22	10	45.5
3948	Coker 40–39	....	29	13	44.8
3949	Coker 40–40	....	13	7	53.8
3950	Coker 40–44	....	16	6	37.5
3951	Coker 40–47	....	22	4	18.2
3694	Ark. 3–28–12–1	....	22	11	50.0

developed by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, in cooperation with the Iowa, North Carolina, Georgia, and Arkansas agricultural experiment stations and the Coker's Pedigreed Seed Company, Hartsville, South Carolina. These selections have been resistant to all other races of smut, and to most races of crown rust to which they have been subjected, and several of them showed such excellent performance that they were distributed to farmers.

Although preliminary results indicated that the Oklahoma smut collection was one of a half dozen known Fulghum-susceptible races of loose smut, it now seems evident that a hitherto unrecorded race of loose smut has been isolated. This race is especially interesting because of its pathogenicity for Victoria, thus far resistant to all known races of both oat smuts.

As shown in table 1, Victoria and Lee were infected 43.7 and 92.5 per cent, respectively. Lee is highly susceptible to many races of both smuts. The 22 selections showed infections by this new race of smut, ranging from 6.6 to 68.0 per cent. This new race may not be widely distributed, and it can only be hoped that many years may elapse before it seriously affects the importance of these new oats in the South.

Strains from other crosses with Victoria that are resistant to other races of smut also were tested for reaction to the new race. Three selections from the cross with Hairy Culberson showed 52.3 to 70.8 per cent of infection when inoculated with the Oklahoma race. On the other hand, selections from crosses of Victoria with Fulgrain, Norton, and Nortex proved resistant. Nortex appears resistant, as also do Ranger and Rustler, which were selected from the Nortex-Victoria cross. Fultex was resistant to Collection 50 in the initial tests, although both Fulghum and Victoria, the parents of Fultex, were somewhat susceptible to the new race. It is probable that these two varieties carry different factors for resistance that combine to evolve resistant lines. Preliminary tests showed that the so-called standard smut-resistant varieties—Bond, Markton, and Navarro—were resistant to this new race. Black Mesdag, susceptible to certain other races, also was resistant. Red Rustproof appears to be resistant.

Further tests are necessary to determine the reaction to this new race of smut of other varieties and selections from Victoria crosses, such as Boone, Tama, and Vicland recently developed, important varieties.

The new race of smut, embraced by Collection 50 from Oklahoma, will be designated as A–30.—GEORGE M. REED, Brooklyn Botanic Garden, and T. R. STANTON, Bureau of Plant Industry, U. S. Department of Agriculture.

THE COMPLEX NATURE OF WHITE-CLOVER MOSAIC

FOLKE JOHNSON[1]

(Accepted for publication May 26, 1941)

INTRODUCTION

White-clover mosaic, a disease that causes streak when transmitted to the garden pea (*Pisum sativum* L.) (13, 14, 24, 25), has been regarded as induced by a single virus, classified by Weiss (18) as Trifolium virus 1. In a recent study (7) on the transmission of viruses by dodder (*Cuscuta campestris* Yunck.), the writer isolated two distinct entities from white clover plants (*Trifolium repens* L.) affected by mosaic. Separation of the two viruses was possible because dodder transmitted only one of them, and the cowpea (*Vigna sinensis* (L.) Endl.) was susceptible only to the other. The virus transmitted by dodder will be referred to as pea-mottle virus and that isolated by means of cowpea will be designated as pea-wilt virus. The present paper presents the results of a study of the properties of these two viruses, their characteristic reactions, and their probable relationships with other legume viruses.

LITERATURE REVIEW

The literature reveals the fact that symptoms of streak in pea may be caused by any one of a number of distinct viruses or virus complexes. Linford (9) noted that pea streak was present in fields from the Atlantic coast to Utah and Montana. He subsequently showed (10) that in Hawaii a similar disease was produced in peas by the virus of pineapple yellow spot, now known to be identical (12, 15) with tomato spotted-wilt virus (*Lethum australiense* H.).[2] Linford's results were confirmed by Whipple (19) and Snyder and Thomas (16), who reported that spotted-wilt virus caused streak in garden peas and sweet peas. Adam (1), in South Australia, obtained results identical with those of Whipple. In a recent communication, Whipple and Walker (20) described two viruses believed to be strains of the common cucumber-mosaic virus (*Marmor cucumeris* H.), both of which caused streak in certain field-grown peas in Wisconsin. Further evidence that strains of the cucumber-mosaic virus were widespread in nature and caused streak in garden peas was given by Zaumeyer (23). Stubbs (17) reported that tobacco-ringspot virus (*Annulus tabaci* H.) caused streak and death of inoculated pea plants. Zaumeyer (22) described an outbreak of streak in garden peas exposed to pea aphids (*Macrosiphum pisi* Kaltenbach) collected from field-grown alfalfa (*Medicago sativa* L.) and believed the disease to be caused by a distinct virus, which he called pea-streak virus 1; Zaumeyer also isolated two strains of alfalfa-mosaic virus (*Marmor medi-*

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[2] The Latin names used in this paper follow the system of nomenclature presented in the Handbook of Phytopathogenic Viruses (5).

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caginis H.), which gave symptoms nearly comparable to streak when used to infect peas. A systemic streak in greenhouse-grown peas inoculated with juice from mosaic-infected white clover was observed by Zaumeyer and Wade (24, 26) and by Pierce (13, 14); while Osborn (11) noted a similar disease caused by the red clover vein-mosaic virus (*M. trifolii* H.). Chamberlain (4) reported a new virus that caused pea streak in New Zealand, and more recently Ainsworth and Ogilvie (2, 3), showed that lettuce-mosaic virus (*M. lactucae* H.) caused streak and death of sweet peas in Great Britain. A single mention of pea streak occurring in China was made by Yu (21). Thus it is known that in addition to the reports of pea streak made by Chamberlain (4) and Yu (21), the viruses of tomato spotted-wilt (pineapple yellow-spot), cucumber mosaic, pea streak, alfalfa mosaic, white-clover mosaic, red-clover vein mosaic and lettuce mosaic caused streak and death to garden peas and sweet peas in various parts of the world.

SEPARATION OF THE VIRUSES FROM THE COMPLEX

Pea-mottle virus was isolated by the following method: 12 white-clover plants infected with the virus complex (Trifolium virus 1) were joined by means of dodder to 12 healthy broad-bean plants (*Vicia faba* L.). Similarly, 8 hop-clover plants (*Medicago lupulina* L.), infected with the virus complex, were connected to 8 healthy hop-clover plants. Nine broad-bean and 5 hop-clover plants became diseased with mosaic during a period of time between 32 and 40 days after connecting them with the diseased plants. The hop-clover plants were mildly mottled and less stunted than those to which they were connected (Fig. 1, *A*). Inoculation of Dwarf Telephone peas with juice from the diseased broad beans and mildly affected hop clover produced symptoms of mosaic (Fig. 1, *B*, b, c) rather than those of streak. The same result was obtained when pea plants were inoculated with juice extracted from dodder that had parasitized infected white clover. On the other hand, inoculations of similar plants with juice from the diseased white clover invariably resulted in streak. The results led to the belief that the white clover was infected with more than one virus, and that dodder had isolated but one constituent of the complex, namely pea-mottle virus.

The second constituent of the complex, referred to as pea-wilt virus, was isolated by inoculation of cowpea with juice from the diseased white clover, since it was found that cowpea plants were not susceptible to infection with pea-mottle virus. Cowpea leaves inoculated with plant juice containing the virus complex developed brown, necrotic, local lesions (Fig. 1, *B*, a). On transfer from infected cowpeas to Hundredfold peas, the virus produced no definite local lesions, but caused wilting and death of the inoculated leaves (Fig. 1, *C*, a). The infected pea plants did not develop streak or chlorotic mottling, but showed only a mild discoloration of the stem; nevertheless, it was easy to demonstrate the presence of virus in the tops of infected plants by inoculation of expressed juice to cowpeas. The pea-wilt virus isolated in this manner, when mixed with the previously isolated pea-

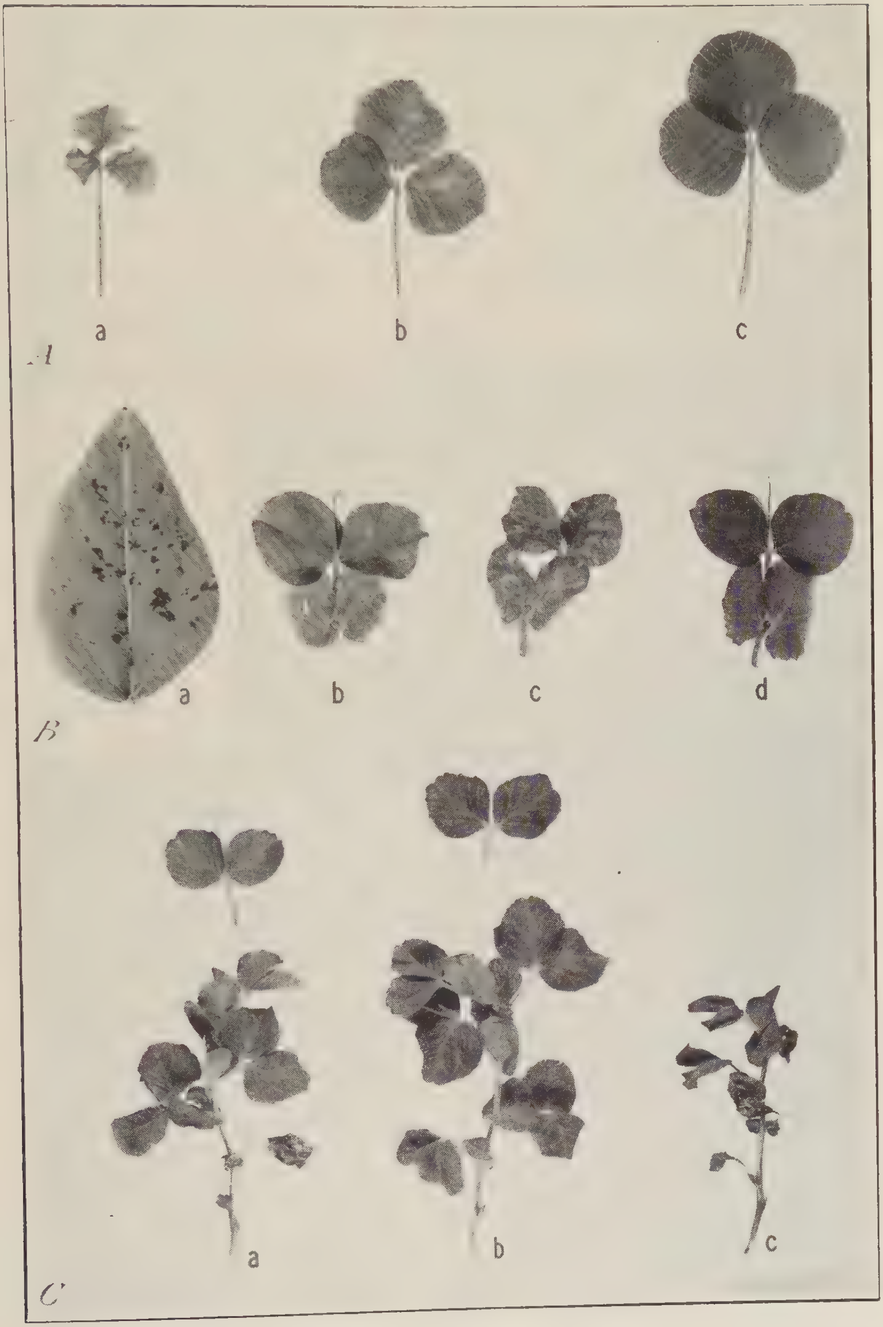

FIG. 1. Symptoms produced by virus complex and separated virus constituents in several plants. *A*, a. Dwarfing and malformation in hop clover caused by virus complex (Trifolium virus 1) from mosaic-infected white clover. b. Mild mottling in hop clover produced by pea-mottle virus. c. Healthy hop-clover leaf. *B*, a. Local necrotic lesions in cowpea produced by pea-wilt virus. b, c. Mosaic in Dwarf Telephone pea caused by pea-mottle virus. Early symptoms at b, later symptoms at c. d. Healthy Dwarf Telephone pea leaves. *C*, a. Death of inoculated leaf resulting in Dwarf Telephone pea from infection of pea-wilt virus. b. Healthy Dwarf Telephone pea plant. c. Streak in Dwarf Telephone pea produced by infection with a combination of pea-mottle virus and pea-wilt virus. (Photographs by Julian A. Carlile.)

TABLE 1.—*Comparative suscept range of pea-mottle virus and pea-wilt virus according to families*

Host plant	Pea-mottle virus		Pea-wilt virus	
	Plants tested[a]	Symptoms[b]	Plants tested	Symptoms
Caryophyllaceae				
Stellaria media (L.) Cyrill.	8/8	M	8/0	
Chenopodiaceae				
Beta vulgaris L. (Sugar beet)	10/0		10/0	
Spinacia oleracea L.	5/5	M	5/0	
Compositae				
Callistephus chinensis Nees	10/0		10/0	
Lactuca sativa L.	10/0		10/0	
Taraxacum officinale Weber	1/0		1/0	
Zinnia elegans Jacq.	20/0		22/0	
Cruciferae				
Barbarea vulgaris R. Br.	1/0		1/0	
Brassica oleracea L.				
var. *capitata* DC.	5/0		5/0	
Raphanus sativus L.	5/0		5/0	
Cucurbitaceae				
Cucumis sativus L.	25/24	YL	25/0	
Gramineae				
Zea mays L.	16/0		19/0	
Leguminosae				
Glycine max Merr.	18/0		22/0	
Lathyrus odoratus L.				
var. Bridal Veil	8/8	M	6/6	m, LN
var. Cardinal	7/7	M	7/7	m, LN
var. Treasure Island	8/8	M	9/4	m, LN
Lens esculenta Moench.	16/16	VC, s	17/17	m, S
Lupinus albus L.	10/10	M, NS	10/1	VC
L. hirsutus L.	10/10	M, NS	10/0	
Medicago lupulina L.	10/10	M	10/10	m
M. sativa L.	10/6	M	10/0	
Melilotus alba Desr.	18/18	M	18/8	m
Phaseolus aureus Roxb.	15/1	VC	14/13	RS, NS
P. vulgaris L.				
var. Early Golden Cluster	15/15	M	15/6	m
var. Great Northern U. of Idaho No. 1	12/10	m	11/1	m
var. Ideal Market	10/0		11/0	
var. Kentucky Wonder	8/8	m	7/0	
var. Navy Robust	7/7	YL, m	9/0	
var. Red Kidney	8/8	YL, m	9/6	m
var. Red Valentine	16/16	M	11/11	m
var. Robust	9/9	YL, m	9/9	m
var. Stringless Refugee	9/9	M	9/5	m
var. Stringless Refugee Green Pod	9/9	M	9/4	m
var. U. S. No. 5 Refugee	12/9	YL, m	12/8	m
var. Unrivalled Wax	12/12	YL, m	12/6	m
Pisum sativum L.				
var. Alaska	15/10	M	17/15	m, LN
var. Dwarf Alderman	30/28	M	30/30	LN
var. Dwarf Telephone	28/26	M	30/30	LN
var. Hundredfold	39/38	M	36/35	LN
var. Laxton Progress	26/20	M	23/23	LN
var. Little Marvel	16/16	M	18/18	LN
var. Nott's Excelsior	18/18	M	20/20	LN
var. Perfection	23/23	M	29/29	LN
var. Potlatch	21/21	M	18/18	LN
var. *arvense* Poir.				
Canada White	20/20	M	19/18	m, LN

TABLE 1.—(*Continued*)

Host plant	Pea-mottle virus		Pea-wilt virus	
	Plants tested[a]	Symptoms[b]	Plants tested	Symptoms
Trifolium hybridum L.	5/5	M	5/5	None
T. incarnatum L.	10/10	m, VC	10/10	VC
T. pratense L.	10/10	M	10/10	m, VC
T. repens L.	10/10	M	10/10	M
Vicia faba L.	25/18	M, ns	23/13	m, RS
V. sativa L.	16/16	m, NS	16/16	m, VC
Vigna sinensis (L.) Endl.	30/0		50/38	m, BLL
Liliaceae				
Lilium formosanum Stapf.	10/0		10/0	
Plantaginaceae				
Plantago lanceolata L.	1/0		1/0	
P. major L.	1/0		1/0	
Polygonaceae				
Rumex acetosella L.	1/0		1/0	
Scrophulariaceae				
Antirrhinum majus L.				
var. Giant Crimson	8/5	M	8/0	
var. Giant White	8/3	M	8/0	
Solanaceae				
Datura stramonium L.	5/0		5/0	
Lycopersicon esculentum Mill.	15/0		15/0	
Nicotiana glutinosa L.	35/0		40/0	
N. tabacum L.	35/0		45/0	
N. rustica L.	5/0		5/0	
N. sylvestris Spegaz. and Comes	20/0		20/0	
Solanum nigrum L.	5/0		5/0	

[a] The numerator indicates the number of plants inoculated, denominator indicates the number of plants diseased.

[b] M = mottling; S = streak; YL = systemic yellow lesions; NS = necrotic spotting; VC = vein clearing; RS = ring spotting; LN = basal leaf wilting and necrosis; BLL = brown local lesions. Similar descriptions with small letters indicate these symptoms were mild.

mottle virus, caused typical symptoms of streak in Dwarf Telephone and Hundredfold peas (Fig. 1, *C*, c).

SUSCEPT RANGE

In order to obtain a better understanding of the relationship between the two viruses, a knowledge of their suscept ranges was needed. An attempt was made to test as far as possible those species and varieties of plants used by other investigators in their work with legume viruses. All plants were grown from seed in a greenhouse held at about 25° C. and fumigated regularly to destroy insects. Plants tested for susceptibility were young and in a stage of rapid growth. The test plants were dusted with carborundum powder and inoculated by the rubbing method. Inoculum was prepared from diseased tissue of young, rapidly growing plants by macerating it in a sterile mortar to which a few drops of tap water were added. A sterile cotton swab on a small stick was dipped in the inoculum and gently rubbed over the plant tissue, which was supported with a sterile pot label. This method of inoculation has been described in detail by Jones (8).

When plants with large leaves were inoculated, sterile gauze pads were used instead of the cotton swabs, and the leaves were supported in the hand. Immediately after inoculation the plants were rinsed with water from a sprinkling can in order to remove any toxic materials. From 2 to 5 leaves were inoculated, depending upon the size and growth habit of the plants. After a suitable incubation period, sub-inoculations were made to peas with juice from the tested host plants.

As is shown in table 1, pea-mottle virus infected plants in the Caryophyllaceae, Chenopodiaceae, Cucurbitaceae, Scrophulariaceae, and Leguminosae, while pea-wilt virus infected plants of the Leguminosae only.

SYMPTOMATOLOGY

Pea-mottle Virus. When pea plants were inoculated with pea-mottle virus, the developing leaves failed to open as readily as in healthy plants; and from 8 to 12 days after inoculation a fine clearing of veins appeared in the young foliage. The large veins were bleached and the network of fine veins stood out in contrast to the adjacent tissues (Fig. 1, *B*, b). Numerous, small, irregular, light-yellow spots were scattered over the youngest foliage. Infected plants were slightly stunted and lighter in color than healthy plants. The first two leaves that developed after the mottling was noticeable were more severely affected than the succeeding ones in which the yellow spots coalesced to form large light-green areas (Fig. 1, *B*, c). The stipules showed the same type of mottle that was found in the leaves. Some varieties of plants outgrew the mottling before blooming. No symptoms were noticeable on the stems; likewise, no apparent effects were observed in the pods or seeds of infected plants.

On bean (*Phaseolus vulgaris* L.), pea-mottle virus produced light-yellow spots and clearing of veins (Fig. 2, *A*, a). These symptoms were uniform on the varieties that were tested. On Alsike clover (*Trifolium hybridum* L.), red clover (*T. pratense* L.) and white clover, light-yellow areas appeared between the veins. The symptoms in alfalfa were distinct, consisting of irregular streaks of yellowing along the veins and adjacent tissues. In some cases dark-green irregular patches of tissue became outlined with light-yellow margins. Infected spinach plants (*Spinacia oleracea* L.) became dwarfed and severely mottled, and infected cucumber (*Cucumis sativus* L.) showed light-yellow secondary lesions (Fig. 2, *B*, b, c).

Pea-wilt Virus. Symptoms produced by this virus in pea were noticeable in from 5 to 8 days following inoculation. The inoculated leaves wilted and died and the petioles shriveled, leaving the dead, dried leaves attached to the stem (Fig. 1, *C*, a). One or more of the adjacent, lower leaves also wilted and died. In most cases the tops of the plants appeared healthy, but in two varieties, Alaska and Canada White, a faint mottling developed and soon disappeared. The stems showed a faint grayish discoloration. Infected plants grew slowly and were dwarfed in comparison with healthy plants.

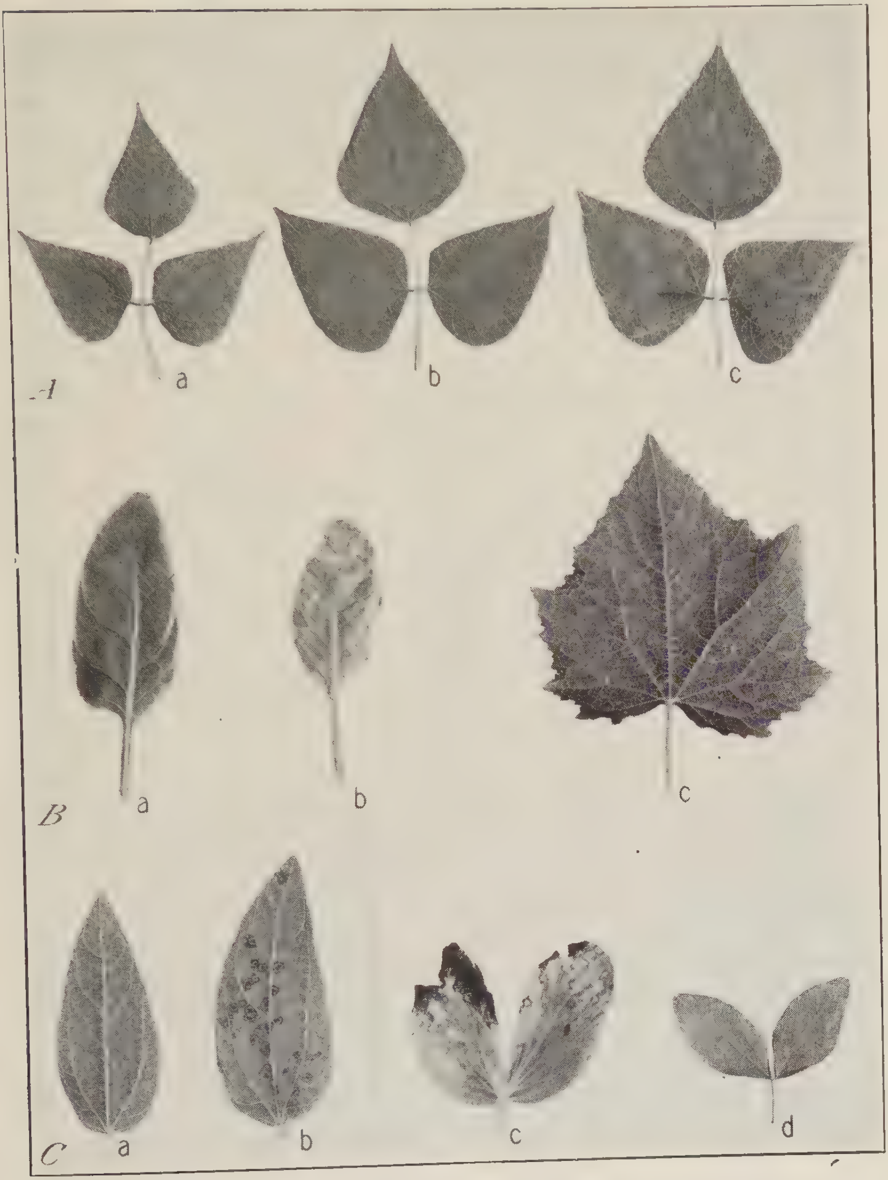

FIG. 2. Symptoms produced by separate virus constituents in several plants. *A*, a. Symptoms in Red Valentine bean caused by pea-mottle virus. b. Healthy Red Valentine bean. c. Diffuse mottle in Red Valentine bean caused by pea-wilt virus. *B*, a. Healthy spinach leaf. b. Mottling in spinach caused by pea-mottle virus. c. Symptoms in cucumber caused by pea-mottle virus. *C*, a. Healthy *Phaseolus aureus* leaf. b. Zonate necrotic spots in inoculated leaf of *P. aureus* caused by pea-wilt virus. c. Ring spotting and necrosis in broad bean caused by pea-wilt virus. d. Mosaic in broad bean caused by pea-mottle virus. (Photographs by Julian A. Carlile.)

In cowpea plants the inoculated primary leaves developed brown, local lesions (Fig. 1, *B*, a), while the trifoliate leaves showed isolated diffuse areas where the veins became slightly bleached. In mung bean (*Phaseolus aureus* Roxb.), necrotic zonate lesions were produced on the inoculated primary leaves (Fig. 2, *C*, b), and in some instances dark necrotic spots developed in systemically invaded leaves. A blotchy mosaic disease was produced in infected bean plants (Fig. 2, *A*, c). Most of the clovers that were infected showed only a very faint mottle, but white clover was noticeably mottled. Alsike clover showed no symptoms of disease, although the plants were systemically invaded by the virus.

HISTOPATHOLOGY

Epidermal strippings and cross sections of stems of Alsike clover, red clover, white clover, hop clover, and several varieties of pea infected with either pea-mottle virus or pea-wilt virus were examined under the microscope for intracellular inclusions. In each case the diseased material was compared with healthy tissue. Water and several other mounting media were used, but iodine-potassium iodide solution, in the proportion of 1 part iodine, 3 parts potassium iodide, and 150 parts water, gave the best results, staining the nuclei brown. Examinations failed to reveal inclusion bodies or unusual crystalline deposits. Calcium oxylate crystals were found in both healthy and diseased plants, but plate crystals, like those found in tobacco and other plants infected with certain viruses, were not observed.

TRANSMISSION STUDIES

In addition to the transmission experiments with dodder, an attempt was made to determine whether or not the pea aphid (*Macrosiphum pisi* Kalt.) was a vector of these viruses under laboratory conditions. Plants of red clover, white clover, hop clover, and Dwarf Alderman peas infected with each virus were caged separately and 30 healthy aphids added to each cage. Healthy plants of each species were treated similarly and used as controls. After a feeding period of 4 days on the diseased and healthy control plants, the aphids were transferred separately from each cage to 6 healthy, caged Dwarf Alderman peas. An attempt was made to place an equal number of insects on each pea plant. The aphids were allowed to feed for a period of 4 days after which they were killed by fumigation, and the plants removed to a greenhouse bench for observation. No symptoms of disease appeared on any of the test plants; likewise, all control plants remained healthy. Since pea-wilt virus did not produce mottling in pea, it seemed possible that the pea plants on which the aphids fed might have been infected without showing definite symptoms. To determine whether this was actually the case, a small portion of leaf tissue was removed from each plant, macerated in a mortar to which pea-mottle virus was added, and used as inoculum for healthy peas. Typical symptoms of pea-mottle developed in the inoculated plants instead of those of streak which would have been the result had the plants been infected with both viruses. This result demonstrated that pea-wilt virus had not been transmitted to peas by the pea aphid.

A second test was made in the following manner: White clover plants infected with each virus were caged separately. One hundred healthy aphids were placed in each cage and allowed to feed 2 days. The aphids from each cage were then transferred separately to 25 individually caged, healthy Dwarf Telephone peas, 4 insects being placed on each plant. All insects were killed after a feeding period of 11 days and the plants removed to a greenhouse for observation. No symptoms of disease developed. As a further check on these plants, a portion of leaf tissue was removed from each plant of each lot, macerated together, and tested for virus by mechanical inoculation of healthy peas. These tests also were negative. The possibility existed that pea-wilt virus was present without showing symptoms of disease; in this event no streak would have appeared if pea-mottle virus had been absent. To test this possibility, leaf tissues from each pea plant in the pea-wilt-virus series were macerated together and pea-mottle virus was added to the plant juice. This mixture was used to inoculate healthy peas. Symptoms of pea-mottle developed in the inoculated plants instead of streak, which would have resulted if both viruses had been present in the inoculum. It is concluded from these results that the pea aphid was not a vector of these viruses.

PROPERTIES OF THE VIRUSES

Thermal Inactivation. Plants infected with each virus were ground separately in a sterile meat chopper, and the juice was extracted by passage through two layers of cheesecloth. The juice was placed in tightly stoppered test tubes (7 × 70 mm.) and completely immersed for 10 minutes in an electrically heated water bath. The water was stirred constantly by means of a stirring rod attached to an electric motor and the temperature was automatically controlled within ± 0.2° C. of the desired temperature. After a 10-minute immersion the test tubes were immediately plunged into ice water, and the cooled cell extract was used to inoculate healthy plants.

Infected plants of Dwarf Alderman, Dwarf Telephone, and Potlatch pea served as source plants for the viruses, and healthy plants of the same varieties were used as test plants. Between 15 and 20 plants were inoculated with each sample and each test was conducted on 3 different occasions. Attempts were also made to use cowpea and mung beans as test plants for measuring activity of the pea-wilt virus, but with erratic results. Therefore, in order to test for pea-wilt virus in heated samples, a small amount of pea-mottle virus was added to each sample after it had cooled. The test plants reacted by production of mottling symptoms when only the pea-mottle virus was present, but by production of streak when pea-wilt virus was also present. It was found that pea-mottle virus was inactivated by exposure for 10 minutes to a temperature of 60–62° C., whereas pea-wilt virus was inactivated by exposure to a temperature of 58–60° C.

Tolerance to Dilution. Infectious plant juice was extracted, as previously reported, and diluted with distilled water in varying proportions up to 1 part in a million. Fifteen to 20 Dwarf Telephone peas were inoculated with each diluted sample. Pea-mottle virus was still infectious at a dilution of 1:10,000, but not 1:100,000, while pea-wilt virus was recovered at a dilution of 1:100,000 but not 1:1,000,000.

Resistance to Aging. Expressed plant juice from plants infected with each virus was stored separately in stoppered bottles at room temperature (about 25° C.) and used as inoculum for 18 to 20 Dwarf Telephone peas. Inoculations were made at 3-day intervals for 3 successive times; subsequent inoculations were made at irregular intervals up to 31 days after extraction. Both viruses withstood aging *in vitro* for 31 days. Neither virus was tested for resistance to aging for longer than this period.

Pea plants infected with pea-mottle virus, pea-wilt virus, and broad bean leaves infected with Zaumeyer and Wade's pea virus 2 were dried at room temperature, held in separate stoppered bottles, and tested for their resistance to aging in dried host tissue. The first inoculation with the dried material was made 7 days after the plants were cut, at which stage the plant tissue was thoroughly dry. A small amount of the dried material was soaked with a few drops of tap water in a sterile mortar, macerated and the plant extract used as inoculum for either Dwarf Alderman or Dwarf Telephone peas. Inoculations were made at 3-day intervals for 3 successive times, after which they were made at irregular intervals. It was found that all the tested viruses withstood aging in dried plant tissue for at least 31 days.

Filterability. Pea plants infected with each virus were separately ground in a meat chopper and the juice was passed through 2 layers of cheesecloth, after which it was centrifuged for 15 minutes at 3300 R.P.M. The liquid was decanted and filtered through a layer of medium-size Celite before passage through a Berkefeld W filter. A 1 cc. sample of the filtered pea juice from each series of plants was added to laboratory broth and thus shown to be free from contamination; subsequently, portions of the filtered plant juice were used as inoculum for 20 to 25 Dwarf Telephone pea plants. In one test, infection was obtained in all plants inoculated with filtered pea-

TABLE 2.—*Comparison of physical properties of pea-mottle virus and pea-wilt virus*

Virus	Thermal inactivation	Tolerance to dilution	Resistance to aging		Filterability
			in vitro	in dry host tissue	
Pea-mottle virus	60–62° C.	1:10,000	At least 31 days	At least 31 days	+
Pea-wilt virus	58–60° C.	1:100,000	At least 31 days	At least 31 days	+

wilt juice, but no plants became diseased when inoculated with pea-mottle filtrate. In a subsequent experiment it was shown that pea-mottle virus passed a Berkefeld W filter and infected inoculated plants. These results prove that both pea-wilt virus and pea-mottle virus pass Berkefeld W filters. The physical properties of the two viruses are summarized in table 2.

DISCUSSION

The fact that Trifolium virus 1 has proved to be a mixture of two viruses was not altogether unexpected, having been suggested by Zaumeyer and Wade (25), who found that the so-called Trifolium virus 1 produced two types of symptoms on many bean varieties. In addition, they reported two different temperatures of virus inactivation and suggested that two viruses might be involved. They, however, did not separate the suspected constituents from the mixture. Pierce (13) found a mosaic infected red-clover plant in the field, the juice from which produced streak when transferred to pea. Inoculations of small-seeded broad bean with juice from the infected red clover resulted in local, necrotic lesions on inoculated leaves followed by a systemic mottling. When extract of the mottled tissue of broad bean was used to inoculate peas, only a mild mottling developed. Pierce considered the mottling virus to be identical with the one he previously had described and named bean virus 2, while the virus that produced local lesions in broad beans was named broad-bean local-lesion virus. Little is known about the latter virus and its reactions in pea. Pierce, however, suggested that when the mottling virus and broad-bean local-lesion virus were combined and used to inoculate pea, streak would be induced, but he cited no definite experimental evidence for this conclusion. It is believed that Pierce and the writer probably worked with the same virus complex, since the writer's original material was collected near Pullman, Washington, a few miles from the locality where Pierce found his infected red clover. This belief is supported by the fact that pea-mottle virus produces a faint mottle in broad beans, while pea-wilt virus causes the production of necrotic ring-spots in the same plants (Fig. 2, *C*, c, d). However, bean virus 2 of Pierce was reported to be non-infectious for white clover and alfalfa and also differed from pea-mottle virus in its resistance to aging *in vitro* and in its tolerance to dilution, thus suggesting that the two viruses are different. The writer believes that Pierce's evidence for considering that bean virus 2 was a component of his red clover virus complex was inconclusive, since it was based only on his observations of the symptoms produced in Stringless Refugee Green beans by his broad-bean mosaic virus. It is the writer's belief that Pierce had the virus complex in red clover that has been classified by Weiss (18) as Trifolium virus 1, but that is shown in this paper to consist of two separate viruses. Pea-mottle virus is similar to the virus that Pierce found to become systemic in broad bean and that produced a mottling in peas, whereas the pea-wilt virus of the writer is probably closely related to Pierce's (13) broad-bean local-lesion virus.

Pea virus 2 of Zaumeyer and Wade (25) resembles pea-mottle virus in the symptoms it produces on many pea and bean varieties. A further similarity between these two viruses is their resistance to drying in host tissue. Other similarities between the two viruses can be found in their suscept range, although they differ somewhat in this respect. One point of difference between pea virus 2 and pea-mottle virus is the fact that pea virus 2 does not produce streak in peas when transferred to these plants in combination with the pea-wilt virus discussed in this paper.

Severe pea-mosaic virus previously described (6) resembles pea-mottle virus on the basis of suscept range, longevity *in vitro* and dried tissue, tolerance to dilution, and heat inactivation. This suggests that these two viruses may be related.

Since it was shown that pea-mottle virus was infectious for plants in 5 different families, the question arises whether this virus is not related to one of the strains of cucumber-mosaic virus that have been found infecting peas (20, 23). Whipple and Walker (20) have shown that 2 cucumber viruses are infectious for a large number of plants in several plant families, including peas and other legumes. Pea-mottle virus has failed to infect corn (*Zea mays* L.), zinnia (*Zinnia elegans* Jacq.), or solanaceous plants, which are generally considered susceptible to infection with cucumber-mosaic virus. Likewise, the symptoms produced by the two viruses studied by Whipple and Walker in peas, beans, and cucumbers differ from those produced by pea-mottle virus.

Not only was it true that pea-mottle virus was infectious for plants in the Caryophyllaceae, Chenopodiaceae, Cucurbitaceae, Scrophulariaceae, and Leguminosae, whereas pea-wilt virus was infectious only for plants in the Leguminosae, but also, in all cases where plants were susceptible to both viruses, a more severe disease was produced by pea-mottle virus than by pea-wilt virus, except in the case of white clover, where similar symptoms were produced by both.

The evidence at hand points to the conclusion that the two viruses discussed in this paper are distinct from each other and that they show characteristics that distinguish them from any of the viruses previously given scientific names according to the system of nomenclature outlined by Holmes (5). In accordance with this system, pea-mottle virus may be referred to as *Marmor efficiens* n. sp., from Latin *efficiens* meaning effective, in reference to the ability of this virus to cause mottling in peas in contrast with the inability of pea-wilt virus to produce such chlorotic symptoms in tested varieties of this host other than Alaska and Canada White. Probable synonyms are: broad-bean-mosaic virus of Pierce (13), which was present in his red-clover-mosaic complex; severe pea-mosaic virus, previously described by Johnson and Jones (6); possibly also pea virus 2 of Zaumeyer and Wade (25). Pea-wilt virus may be referred to as *M. repens* n. sp. The specific name is taken from Latin *rĕpens* (not from *rēpens*) and means unlooked for, in reference to the unexpected discovery of a second virus in the original complex. Broad-bean local-lesion virus of Pierce (13) may be considered as a probable synonym.

SUMMARY

It has been shown that white-clover mosaic, a disease previously regarded as caused by a single virus classified as Trifolium virus 1, was actually induced by a mixture of two distinct viruses: namely, pea-mottle virus and pea-wilt virus. Their separation was accomplished because pea-mottle virus alone was transmitted by dodder (*Cuscuta campestris*), while pea-wilt virus infected cowpea (*Vigna sinensis*), a plant resistant to pea-mottle virus.

When pea-mottle virus in combination with pea-wilt virus was transferred to peas, streak was produced and resulted in death of the plants in a manner similar to that produced by the Trifolium virus 1 complex.

Pea-mottle virus alone produced a systemic mosaic disease when transferred to several pea varieties, and was infectious for plants in the Caryophyllaceae, Chenopodiaceae, Cucurbitaceae, Scrophulariaceae, and Leguminosae. In the tests conducted, pea-wilt virus was infectious for plants in the Leguminosae only, and produced no mottling in pea varieties, except Alaska and Canada White, where a very mild mosaic was produced. In general, plants infected with pea-mottle virus were more severely affected than when similar plants became infected with pea-wilt virus. No intracellular inclusion bodies could be detected in plants infected with either virus, and no virus transmission was obtained by allowing the pea aphid (*Macrosiphum pisi*) to feed on infected and healthy pea plants in succession.

Pea-mottle virus was inactivated by exposure for 10 minutes to a temperature of 60–62° C., whereas pea-wilt virus became inactive between 58–60° C. Pea-mottle virus was recovered in a dilution of 1:10,000 in water, and pea-wilt virus in a dilution of 1:100,000. Both viruses withstood aging *in vitro* and in dried host tissues for at least 31 days and were filterable through a Berkefeld W filter.

The name **Marmor efficiens** n. sp. is suggested for pea-mottle virus and **M. repens** n. sp. for pea-wilt virus.

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NEW PHYSIOLOGIC RACES OF TILLETIA TRITICI AND T. LEVIS[1]

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INTRODUCTION

In a previous paper the writers (9) discussed the status of physiologic-race identification in *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn and emphasized the need for a standard system on which to base the identification of races, particularly as related to the breeding and distribution of resistant varieties of wheat in the United States. At that time 11 races of *T. tritici* and 8 of *T. levis* were classified on the basis of the resistant (R), intermediate (I), and susceptible (S) reaction of certain winter- and spring-wheat varieties. Since the publication of that paper, additional collections of *T. tritici* and *T. levis* from all of the principal wheat-growing States have been tested and 5 additional races of these fungi have been identified and numbered, consecutively, in accordance with the original system. Also, studies and observations have been made on race-differentiating characters other than pathogenicity. The results of these studies and the extended race classification are here presented.

MATERIALS AND METHODS

All of the previously classified physiologic races of *Tilletia tritici* and *T. levis* (9) and many collections of both species obtained from commercial wheat fields in the principal wheat-growing sections of the United States were used in these studies. The differential varieties were the same as those used in the former studies (9), except that Martin (C.I.[3] 4463) and White Odessa (C.I. 4655) were added to the winter-wheat group, and Mindum (C.I. 5296) was omitted from the spring-wheat group. Hybrid 128 was used throughout as a susceptible winter-wheat check.

The tests on winter wheats were made at Pullman, Washington, and those on spring wheats at Bozeman, Montana. In 1940 an additional nursery was grown at Aberdeen, Idaho. The previously described (9) technique for conducting the experiments and analyzing the data for race identification was employed in these studies. Race differentiation by means other than pathogenicity is mainly a matter of observation, and any special techniques used in this phase of the investigations are described along with the presentation of results.

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[1] Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Agricultural Experiment Stations of Idaho, Montana, Oregon, Utah and Washington.

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[3] C.I. denotes accession number of the Division of Cereal Crops and Diseases.

EXPERIMENTAL RESULTS

New Physiologic Races

Three new races of *Tilletia tritici* and 2 of *T. levis* have been identified on the basis of results obtained from pathogenicity tests made during the 4 years, 1937–1940. The percentages of infection obtained on the differential varieties with these races each year are summarized in table 1; and the reaction of the differential varieties (host testers) to these races and those previously classified is shown in table 2. Although the identity of the new races is based primarily on the reaction of the winter wheats, some of them differ also in pathogencity on the spring wheats. As shown in tables 1 and 2, T–12 is differentiated by the susceptibility of Hohenheimer, Albit, and White Odessa, and the intermediate reaction of Martin. This is the only race that infects all of these varieties. Race T–13 is differentiated by the susceptibility of Ridit, Hussar, Albit, and White Odessa, and, likewise, this is the only race that infects all of these particular varieties (Tables 1 and 2). On the other hand, T–14 is differentiated by the susceptibility of Albit and White Odessa only (Table 2), whereas all the other races that infect these two varieties also infect one or more other varieties. The identity of L–9 is based on the susceptibility of Ridit and Albit, while L–10 is differentiated by the intermediate reaction of Ridit (Tables 1 and 2). In this analysis it is notable that four of the new races can be identified by the reaction of the host testers used in previous studies, while the identity of one of these races (T–14) necessitates the use of Martin and White Odessa also.

As shown in table 1, there was some degree of variability in the pathogenicity of the new races in the different seasons. Relatively low percentages of infection were obtained in 1938 with T–12, T–13, T–14, and L–9, due, apparently, to unfavorably high temperature during the infection period. Percentages of infection with these races in that year are, therefore, omitted from the averages. Apparently, these conditions did not affect the virulence of L–10, and the percentages of infection with this race are included in the averages.

These data support Aamodt's (1) suggestion that there are differences in the optimum temperature requirements for infection by different races. Generally, however, relatively low temperature favors infection by the bunt fungi; and, with the exception referred to, there is a notable consistency in the reaction to the races shown in table 1, particularly of the varieties that identify the various races. For example, the identity of T–12 is based on the S reaction of Hohenheimer and Albit, and the percentage of infection on both varieties is well within that infection class each year. In the case of T–13, which is identified by the S reaction of Ridit and Albit, the percentages of infection on Albit are well within that infection class, while on Ridit they are just slightly above the minimum for the susceptible class. Nevertheless, it seems noteworthy that for three consecutive years the S

TABLE 1.—*Percentage of bunt produced on differential varieties by three physiologic races of Tilletia tritici and two of T. levis*

Species	Race No.	Year	Smutted heads in										
			Hybrid 128 (C.I. 4512)	Ridit (C.I. 6703)	Oro (C.I. 8220)	Hohenheimer (C.I. 11458)	Hussar (C.I. 4843)	Albit (C.I. 8275)	Martin (C.I. 4463)	Wh. Odessa (C.I. 4655)	Ulka (C.I. 11478)	Marquis (C.I. 3641)	Canus (C.I. 11637)
			P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Tilletia tritici	T–12	1937	89.0	0.7	1.4	73.0	8.0	92.0	....	....	73.0	11.0	0.1
		1938[a]	85.0	1.0	2.0	10.0	3.0	59.0	....	....	86.0	9.0	0.0
		1939	....	0.0	0.0	69.0	1.0	75.0	23.0	79.0	....	....	....
		1940	95.0	2.0	1.0	58.0	1.0	76.0	31.0	89.0	96.0	18.0	0.0
		Av.	92.0	1.3	1.2	66.6	3.3	81.0	27.0	84.0	85.0	12.6	0.0
Do	T–13	1938	70.0	15.0	1.0	0.0	8.0	33.0	....	....	....	15.0	23.0
		1939	92.0	43.0	4.0	0.0	48.0	85.0	....	....	....	....	....
		1940	66.0	45.0	1.0	0.0	55.0	71.0	0.0	85.0	18.0	26.0	27.0
		Av.	79.0	44.0	2.5	0.0	51.5	78.0	0.0	85.0	....	20.5	25.0
Do	T–14	1938	45.0	2.0	3.0	0.0	0.1	24.0	2.0	65.0	....	....	....
		1939	95.0	0.3	1.0	0.0	3.0	76.0	3.0	86.0	....	....	....
		1940	95.0	1.0	1.0	0.0	9.0	86.0	0.0	93.0	7.0	2.0	0.0
		Av.	95.0	0.6	1.0	0.0	6.0	81.0	1.5	89.5	7.0	2.0	0.0
Tilletia levis	L– 9	1937	67.0	52.0	9.0	1.0	20.0	45.0	....	....	....	....	....
		1938	76.0	52.0	6.0	0.0	6.0	23.0	....	....	....	....	....
		1939	88.0	38.0	4.0	0.0	31.0	66.0	....	....	....	....	....
		1940	87.0	63.0	1.0	0.0	58.0	64.0	0.0	82.0	51.0	23.0	31.0
		Av.	80.6	50.6	4.6	0.3	36.3	58.3	0.0	82.0	51.0	23.0	31.0
Do	L–10	1938	92.0	46.0	9.0	0.0	0.0	0.0	....	....	90.0	16.0	18.0
		1939	64.0	22.0	13.0	0.0	0.0	0.0	....	....	....	....	....
		1940	90.0	38.0	7.0	0.0	0.0	0.0	0.0	0.0	99.0	26.0	37.0
		Av.	82.0	34.3	9.6	0.0	0.0	0.0	0.0	0.0	94.5	21.0	27.5

[a] The data for 1938 are not included in the averages, except for L–10, which apparently was not affected by conditions that were unfavorable for infection by other races.

TABLE 2.—*Reaction of physiologic races of Tilletia tritici and T. levis to the differential varieties*

Race No.	Hybrid 128	Ridit	Oro	Hohenheimer	Hussar	Albit	Martin	White Odessa	Ulka	Marquis	Canus	Mindum
T– 1	S[a]	R	R	R	R	R	R	R	S	I	R	R
T– 2	S	R	R	R	R	R	R	R	S	R	R	S
T– 3	S	R	R	R	R	R	R	R	S	S	S	I
T– 4	S	R	R	R	R	I	S	S	S	S	R	I
T– 5	S	R	R	R	R	I	S	S	S	S	S	I
T– 6	S	R	R	R	R	S	S	S	S	S	R	I
T– 7	S	R	R	R	I	S	S	S	S	S	I	I
T– 8	S	R	R	R	S	S	S	S	S	S	S	I
T– 9	S	R	R	I	R	R	R	R	S	I	R	I
T–10	S	R	R	S	R	R	R	R	R	I	R	R
T–11	S	S	R	R	R	R	R	R	I	S	S	I
T–12	S	R	R	S	R	S	I	S	S	R	R	...
T–13	S	S	R	R	S	S	R	S	...	I	I	...
T–14	S	R	R	R	R	S	R	S	S	R	R	...
L– 1	S	R	R	R	R	R	R	R	S	I	R	I
L– 2	S	R	R	R	R	R	R	R	S	S	R	I
L– 3	S	R	R	R	R	R	R	R	S	S	S	I
L– 4	S	R	R	R	R	S	S	S	S	I	R	I
L– 5	S	R	R	R	R	S	S	S	S	S	S	I
L– 6	S	R	R	R	I	S	S	S	S	S	S	I
L– 7	S	R	R	R	S	S	S	S	S	I	S	I
L– 8	S	R	S	R	R	R	R	R	S	S	S	I
L– 9	S	S	R	R	I	S	R	S	...	...	...	...
L–10	S	I	R	R	R	R	R	R	...	...	...	...

[a] R = Resistant (0–10 per cent infection); I = Intermediate (11–40 per cent infection); S = Susceptible (41–100 per cent infection).

reaction on Ridit was maintained by a slight margin. The S reaction of Albit and White Odessa and the R reaction of Martin, Hussar, and Ridit differentiates T–13 from the other races; and the percentages of infection produced by this race, shown in table 1, are well within their respective classes.

Slight discrepancies are apparent in the results with L–9, which is identified by the S reaction of Ridit and Albit. The percentage of infection on Ridit was slightly below the minimum for the susceptible infection class in 1939 but well above the minimum in the other 3 years. Also, the reaction of Hussar, although not important in the identification of this race, averaged intermediate (I) over a 3-year period but was susceptible (S), in 1940. These results, and those obtained with other races, indicate that the intermediate (I) reaction class is highly variable and, consequently, may not be entirely reliable in race identification, particularly when the percentage of bunt usually obtained is near the minimum or the maximum for this class. Nevertheless, there is a definite intermediate reaction, as exemplified by T–9 on Hohenheimer, which it seems desirable to recognize, even though several years' results may be required to establish such a reaction.

The original source of the inoculum of each of the 5 new races of *Tilletia* *tritici* and *T. levis* is shown in table 3. It will be noted that T–12 came from Oregon, T–13 and L–10 from Washington, and T–14 and L–9 from Idaho. The original source of T–13 is of particular interest and is described later in this paper in discussing the stability of physiologic races.

TABLE 3.—*Original source of the inoculum of the five new races of Tilletia tritici and T. levis*

Species	Collection No.	Race No.	Source
Tilletia tritici	268	T–12	From a commercial wheat field in Umatilla Co., Oreg. Collected in 1934 by J. F. Martin who made preliminary tests in 1935 and 1936 on the differential hosts at the Pendleton Field Station, Pendleton, Oreg.
	329	T–13	A selection of T–11 from the variety Albit in the physiologic race nursery at Pullman, Wash., 1937. Collected by C. S. Holton and H. A. Rodenhiser.
	334	T–14	From a commercial wheat field near Cottonwood, Idaho. Collected in 1936 by W. M. Bever who made preliminary tests on the differential hosts at the Idaho Agricultural Experiment Station, Moscow, Idaho.
Tilletia levis	331	L– 9	From a commercial field of Ridit near Lewiston, Idaho. Collected in 1936 by C. S. Holton.
	92	L–10	From an increase plot of Ridit on the Washington Agricultural Experiment Station, Pullman, Wash. Collected in 1932 by C. S. Holton.

Distinguishing Characters of Physiologic Races Other than Pathogenicity

It is recognized that physiologic races of *Tilletia tritici* and *T. levis* may exhibit differences in characteristics other than pathogenicity. These differences, which may be either morphological or physiological, include such characteristics as size and shape of bunt balls, size of chlamydospores, prominence of spore wall reticulations, spore size and color, relative capacity for stunting the host, and the tendency for partial smutting of the wheat spikes. Studies have been made of these characters of some or all of the races now recognized and the results are here presented.

One of the principal secondary criteria for distinguishing between races of the bunt fungi is characteristics of the bunt balls. The bunt ball characteristics of the 24 races of *Tilletia tritici* and *T. levis* on both spring and winter wheat varieties listed in table 2 were studied. Noteworthy differences in size and shape of the bunt balls were observed in certain races but none of these was essentially different from those reported by other workers (3, 4, 6, 12).

Bunt balls of different physiologic races may differ also in hardness and in rate of water absorption. Those of the so-called dwarf bunt are characteristically small, hard, and almost spherical (15). Usually, individual bunt balls of this race are sufficiently hard to be difficult to crush with the fingers. The chlamydospores, however, appear very dry and powdery in contrast to the oily nature of those of other races. This difference probably accounts for the rapidity with which the balls of the dwarf bunt absorb water. Repeated tests have shown that the pericarp ruptures and the spores are exuded, almost invariably, within a few minutes after bunt balls of this race come in contact with water, while those of other races require from several hours to several days to rupture and exude spores, unless their pericarps are cracked. In tests with 100 bunt balls of each of 6 races of *Tilletia tritici,* approximately 75 per cent were intact after 18 hours. During this time the average weight of each bunt ball had more than doubled due to absorbed water. In similar tests with the same number of bunt balls of each of four races of *T. levis* approximately 90 per cent of those of each race were intact at the end of 18 hours, and the average weight of each was more than doubled by water absorption. In tests with many balls of the dwarf bunt, represented by specimens from different localities, the majority broke within 2 or 3 minutes and only one was observed that required longer than 10 minutes for the pericarp to break after coming in contact with water. In this case, 58 minutes elapsed before the spores began to exude. Obviously, therefore, the dwarf bunt race of *T. tritici* is distinctly different from other races of this species and of *T. levis* in respect to water-absorptive properties of the bunt ball. The exact nature of this difference has not been determined; possibly it is connected with a difference in the oil content of the spores.

Prominence of the reticulations of the chlamydospore walls and size of the chlamydospores are two of the more common morphological characters by which physiologic races of the bunt fungi may be distinguished. Such differences have been reported by several investigators (3, 6, 12, 15). The writers also have observed that some of the races studied differ in these characteristics. For example, chlamydospores of T–8 appear almost smooth, because of their extremely shallow reticulations, whereas those of the dwarf bunt seem spiny because of their prominent reticulations. Intergrading types, also, exist, typically represented by the reticulations of T–9 and T–10, thus completing a series from the T–8 type to that of the dwarf bunt. Apparent differences between reticulations of the spores of other races have been observed, but, because of the intergrading types, finer distinctions than those described above usually cannot be made with certainty. Similar differences were observed by Gassner (5) who established *Tilletia tritici* var. *intermedia* on the basis of the intermediate character of the reticulations of the chlamydospores. Spore size is considered to be of limited value in distinguishing between races of the bunt fungi. Usually measurements of many spores are necessary so that statistical analysis may be applied in order to show significant differences. Differences in spore size among different races have been demonstrated by several workers (6, 11, 13). The writers measured the diameter of 100 chlamydospores of each of 12 races of *Tilletia tritici* and 8 of *T. levis*. In the former species the diameter of the spores ranged from averages of 17.1 μ in T–3 to 19.7 μ in T–10. In *T. levis* the average spore diameters ranged from 16.3 μ in L–1 to 17.5 μ in L–7. These data indicate that the spores of the *T. levis* races, on the average, are smaller than those of the *T. tritici* races. Obviously, therefore, some races of the bunt fungi differ in size of chlamydospores; for the most part, however, these small differences cannot be used to any particular advantage in the differentiation of races.

Chlamydospore color, also, is recognized as a possible means of distinguishing between races of *Tilletia tritici* and *T. levis*. On this basis Spangenberg and Gutner (13) described 4 races of *T. tritici* and 3 of *T. levis*. In the former species the chlamydospores were classified as dark-brown, light-brown, typical, and whitish-brown, while in the latter they were classified as dark-brown, light-brown, and greyish-brown. In studies made by the writers on 25 races of both species, it was found that, in general, the spores of *T. levis* were of lighter color than those of *T. tritici*. One race of *T. levis* (L–4, represented by Collection 250 from Griffin, Ind.) was observed to have spores conspicuously lighter in color than all of the other races. But with this exception, race differentiation on the basis of spore color was unsuccessful.

Physiologic races may differ also in their ability to stunt the host, as shown by other investigations (6, 8, 15) and by the writers. Previously (6), it was reported that T–9 and T–10 differed in their capacity for stunting Hybrid 128 and Hohenheimer. Subsequent observations have confirmed this. Also, collection No. 258 of *Tilletia tritici,* which has about the same pathogenicity as T–1, is readily distinguished from T–1 and other races by its ability to stunt Hybrid 128 and Ulka. Dwarf bunt causes the greatest stunting of any known race. Differences, also, were observed in the stunting caused by *T. levis*, notably L–8. Race identification by this means, however, is limited to the extreme types cited above.

Studies also were made to determine whether reduction in height results from reduction in length of the internodes, as reported by Barrus (2) and Mourashkinsky (7), or in number of internodes, as found by Viennot-Bourgin (14). The results of these studies are presented in table 4. It will be noted that there was no significant difference in the number of internodes on infected and bunt-free plants of the same variety. Marked differences were exhibited, however, in the internode length of infected and bunt-free plants. In Hybrid 128, the internodes of the bunt-free plants averaged 17.2 cm. in length, while those of plants infected by T–13 and collection No. 258 averaged 12.8 and 6.9 cm., respectively. With Albit and Triplet, comparable results were obtained. Thus, in these varieties reduction in height of plants infected by these races is attributable to shortening of the internodal length, which agrees with the results reported by Barrus (2) and Mourashkinsky (7).

Incomplete smutting of infected heads also may be a distinguishing characteristic of physiologic races. Collection 13 of *Tilletia tritici* and T–8 both exhibit this phenomenon on Hohenheimer and Hosar, respectively. A summary of the data obtained from a study of heads partly smutted by these two races is presented in table 5. On Hohenheimer, infected by collection No. 13, 90.4 per cent of the infected heads were partly smutted and only 9.6 per cent totally so. Usually there were only one or a few bunt balls per head. Different degrees of incomplete smutting of Hohenheimer by this race are shown in figure 1. For the most part, however, there were fewer bunt balls than sound kernels, the respective percentages being 60.2 and 39.8 (Table 5). As shown further in table 5, although 21.9 per cent of the heads were infected, only 8.7 per cent of the kernels were destroyed. Similar results were obtained with T–8 on Hosar (Table 5). Both of these races produced only totally-smutted heads on Hybrid 128.

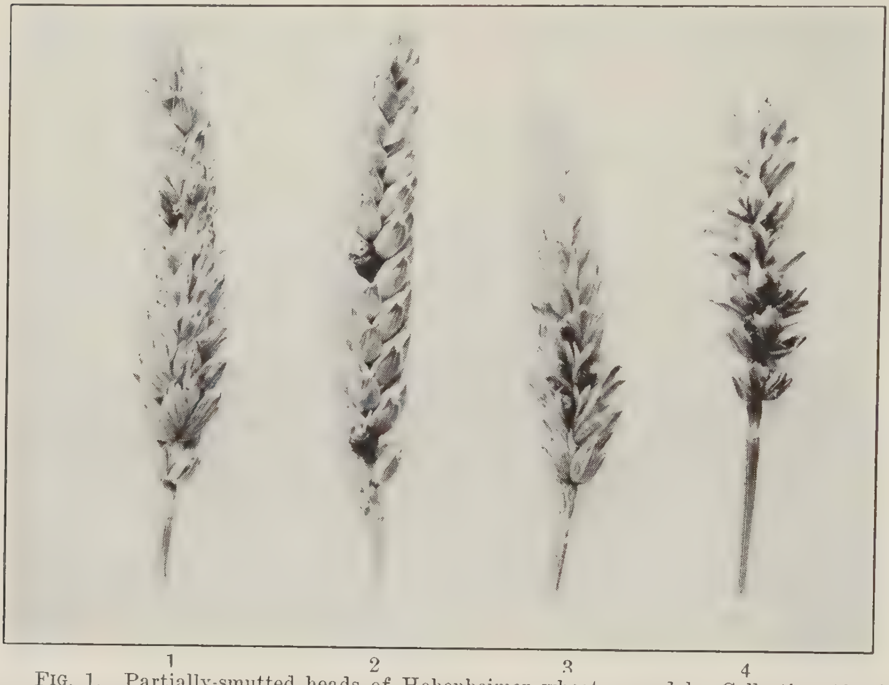

FIG. 1. Partially-smutted heads of Hohenheimer wheat caused by Collection 13 of *Tilletia tritici*. (1) One bunt ball; (2) Two bunt balls; (3) Five bunt balls; (4) Completely smutted head.

The pathogenicity of collection No. 13 of *Tilletia tritici* on the differential varieties places it in the category of T–9, but its incomplete smutting proclivity on Hohenheimer readily distinguishes it from that race, which produces totally smutted heads on this variety.

Two other criteria for the separation of races of the bunt fungi are recognized. One of these is the capacity to intensify the purple pigmentation in the glumes of Ulka. This is especially true of T–8, T–10, and T–11,

TABLE 4.—*Average number and length of internodes in bunt-free and infected culms of three varieties of wheat*

Variety	Race	Number of culms	Internodes per culm	Length of internodes
			No.	*cm.*
Hybrid 128	T–13	100	5.8	12.8
	Collection 258[a]	100	5.5	6.9
	Bunt-free	100	5.8	17.2
Albit	T–13	100	5.8	13.7
	Bunt-free	100	5.7	16.9
Triplet	Dwarf bunt	124	4.6	7.6
	Bunt-free	95	5.0	18.3

[a] Collection No. 258 of *Tilletia tritici*. No race number has been assigned.

TABLE 5.—*Summary of data on incomplete smutting caused by two physiologic races of Tilletia tritici on two wheat varieties*

Variety	Race	Smutted heads	Heads smutted		Kernels		Smutted	
			Partly	Totally	Sound	Smutted	Heads	Kernels
		Number	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Hohenheimer (C.I. 11458)	Coll. 13[a]	104	90.4	9.6	60.2	39.8	21.9	8.7
Hosar (C.I. 10067)	T–8	113	95.0	5.0	62.8	37.2	43.1	16.0

[a] Collection 13 of *Tilletia tritici*. No race number has been assigned.

and to a lesser extent T–3 and T–5. Only race 9 of *Tilletia levis* expressed this characteristic. The other criterion is the capacity to cause excessive tillering of infected plants. This is particularly characteristic of the dwarf bunt race (12). Although definite counts have not been made of the number of tillers produced on plants infected by this and other races, enough observations have been made under different environmental conditions and on different varieties to establish the fact that this race is clearly different in its capacity to stimulate excessive tillering.

STABILITY OF PHYSIOLOGIC RACES

In the bunt fungi the term physiologic race is used to designate chlamydospore populations that differ from each other clearly, and fairly consistently, in one or more ways, but mainly in pathogenicity. Variability in the pathogenicity of a race may result either from environmental influence on the host or pathogen (1, 10) or from changes in the genetic constitution of the chlamydospores. Since the chlamydospores are diploid, considerable variation may possibly occur in one or more of the differentiating characters in succeeding generations, unless the spores are homozygous, in which case variability might arise as a result of mutation. This is an important consideration in race classification, as the identification of

TABLE 6.—*Summary of data indicating heterozygosity of the chlamydospores of race T–11, on the basis of the reaction of differential varieties in 1935–1940*

Collection No.	Race	Year	Inoculum from	Smut percentages in							
				Hybrid 128	Ridit	Oro	Hohenheimer	Hussar	Albit	Martin	White Odessa
	T–11	1935	Ridit	90.0	55.0	1.0	0.0	0.0	2.0	......	. . .
	ʻʻ	1936	ʻʻ	77.8	56.4	2.8	0.0	0.0	11.2	......	....
	ʻʻ	1937	ʻʻ	75.3	44.1	4.2	0.0	16.7	27.4	......	....
	ʻʻ	1938	ʻʻ	59.0	50.0	0.0	0.0	0.0	9.0	......	....
329	ʻʻ	1938	Albit	70.0	15.0	1.0	0.0	8.0	33.0	......	....
330	ʻʻ	1938	Hussar	49.0	9.0	0.0	0.0	7.0	19.0	......	....
	ʻʻ	1939	Ridit	....	52.5	2.0	0.0	4.8	15.1	......	....
329	T–13	1939	Albit	92.0	43.0	4.0	0.0	48.0	85.0	......	....
330	ʻʻ	1939	Hussar	....	58.0	6.3	0.0	54.0	81.5	......	....
	T–11	1940	Ridit	85.0	47.0	2.0	3.0	0.0	1.0	1.0	5.0
329	T–13	1940	Albit	66.0	45.0	1.0	0.0	55.0	71.0	0.0	85.0

new races is contingent upon a relatively high degree of constancy in the characteristics of known races. The writers have attempted to determine, insofar as is possible, the stability under field conditions of the physiologic races of *Tilletia tritici* and *T. levis* classified in table 2.

Lack of purity in chlamydospore populations of *Tilletia tritici* and *T. levis* may be due either to mechanical mixture, in which case genotypically different chlamydospores would be included in one collection, or to heterozygosity of the spores, resulting from hybridization. Obviously, mechanical mixtures make hybridization possible, and a heterozygous condition may result. In race identification, either condition is a complicating factor that might be obscured for a number of years. Fortunately, this factor apparently is not often encountered, as is indicated by the fact that almost all of the races identified by the writers have remained remarkably constant in pathogenicity on the winter-wheat differential varieties for several years in tests at Pullman. However, at least one race, T–11, was not constant (Table 6). This race is characterized by the susceptibility (S) of Ridit and the resistance (R) of the other winter-wheat differential varieties (Table 2). The typical reaction was obtained in 1935, but, in 1936, the percentage of infection on Albit was slightly above the upper limits of the resistant (R) class; and, in 1937, there was a further increase in virulence on Albit and also on Hussar. Studies were undertaken to determine whether the apparent change in pathogenicity of T–11 was attributable to mechanical mixture with another race, to a change in the genetic constitution, or to the influence of seasonal differences in the environment.

In 1937, inoculum was taken from Ridit, Albit (Collection 329), and Hussar (Collection 330) and used to inoculate the winter-wheat differential varieties. Although the infection percentages for 1938 were relatively low, because of unfavorable conditions for infection, they indicated that 2 distinct types of pathogenicity were represented in the 3 collections. Again, inoculum was taken from the 3 varieties, as indicated above and shown in table 6, and tests were made in 1939. The results show clearly that T–13, a new race, characterized by the susceptible (S) reaction of Ridit, Hussar, and Albit, was separated from the original T–11. The reaction of the differential varieties to T–13 was the same in 1940 as in 1939. Its contrast to the T–11 reaction is shown by the results obtained with inoculum of T–11 from another source.

The exact origin of T–13 cannot readily be explained by the data at hand. It appears, however, that the change was of a genetic nature, due either to segregation of factors for pathogenicity in heterozygous spores of the original T–11 or to mutation.

DISCUSSION

The importance of physiologic specialization of *Tilletia tritici* and *T. levis* as a factor in the problem of control of bunt of wheat through resistant varieties is too well understood to require elaboration here. Obviously, however, the identification of new races has a direct relation to the complexity of this problem. For example, prior to the identification of T–12, no race capable of infecting both Albit and Hohenheimer was known, although each of these varieties was susceptible to other races. Therefore, a cross between these two varieties might have been expected to produce segregates that would be resistant to all known races. The discovery of T–12, however, eliminated that expectation because of the common susceptibility of Albit and Hohenheimer to this race. Similarly, Albit and Ridit are now known to have common susceptibility to races T–13 and L–9, whereas, formerly, this situation was not recognized. In other words, an increase in the number of known races serves to reduce the number of combinations of apparently highly bunt-resistant varieties. Consequently, it seems desirable to identify and recognize the greatest possible number of existing races of *T. tritici* and *T. levis* and thereby enhance the production of varieties with the highest possible resistance.

The differentiation of physiologic races of the bunt fungi by criteria other than pathogenicity seems to have limited application, insofar as the majority of races used in these studies are concerned. Certain notable examples were observed in which races were readily distinguished by bunt-ball characters, chlamydospore markings, effect on host development, and other means, but these differences apparently have no direct relation to the problem of bunt control. In the final analysis, at least from the practical point of view, pathogenicity is the most important consideration; consequently, this criterion remains the primary basis for race identification.

The reliability of a standard system for the identification of physiologic races of *Tilletia tritici* and *T. levis* depends largely on the stability of races after they have been identified and numbered. In other words, if the pathogenicity of a race is to be of any value for comparison with new, unidentified races, it should remain fairly constant in its reaction to a given set of differential varieties. The possibilities for hybridization and mutation in the bunt fungi are well known; because of these possibilities considerable variability in pathogenicity and other characters of a given race might be expected, unless the original inoculum consisted of homozygous spores. In dealing with the large number of races and collections used in these studies, efforts were made to obtain reasonably constant pathogenic reactions over a period of three years before designating a collection as a race. That a high degree of success was obtained seems to be indicated by the fact that of 19 races identified in 1937 only one (T–11) failed to maintain its identity. In this case a new race (T–13) was derived from a known race and there is evidence that the original race was retained. As already pointed out, the origin of T–13 probably was due to the segregation of factors for pathogenicity in heterozygous spores or to mutation.

It would seem from these results with T–11 that a race may appear to be essentially pure throughout a series of tests and then break up into two or more races.

SUMMARY

Five new races of *Tilletia tritici* and *T. levis* are described and numbered, thus bringing the total number of known races to 24, 14 of the former and 10 of the latter species.

Some of the races of *Tilletia tritici* and *T. levis* are distinguishable by one or more of the following criteria, other than pathogenicity: bunt-ball size, shape, and water absorptive properties; chlamydospore size, echinulation, and color; capacity to stunt the hosts and to stimulate excessive tillering of infected plants; incomplete smutting and capacity to intensify pigmentation of the glumes.

Race T–11 gave rise to T–13, one of the new races, thus indicating that some races are pathogenically unstable. Most of the races, however, proved highly stable in the tests on winter wheats at Pullman, Wash.

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PHYSIOLOGICAL STUDIES ON TWO SPECIES OF DIPLODIA PARASITIC ON CORN[1]

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INTRODUCTION

The two species of the genus *Diplodia* that occur most commonly on corn, *Zea mays* L., are *D. zeae* (Schw.) Lév. and *D. macrospora* Earle. Macroscopically, these fungi are very similar; but there is a marked difference in the size of the pycnospores, the spores of *D. macrospora* measuring 70–80 × 6–8 μ, while those of *D. zeae* measure 25–33 × 5–6 μ. In contrast to the morphological resemblance of the fungi and the similarity in appearance of decayed corn ears, the organisms exhibit distinct physiological differences. In all their contrasting characters, at least in this country, *D. zeae* seems the more vigorous. The two fungi also differ greatly in geographical range. *D. zeae* apparently occurs wherever corn is grown and causes considerable damage to the crop as a stalk and ear rot and at times as a seedling blight. In the United States *D. macrospora* has been reported chiefly from the southeastern states of Florida, Louisiana, Alabama, North Carolina, South Carolina, and Tennessee (10). *D. macrospora* also has been found in Brazil, Argentine, and Africa (7, 10, 20).

Both Miss Johann (7) and Miss Kinsel (8) have reported that *Diplodia zeae* grows more vigorously on synthetic media than does *D. macrospora*. Stevens (18) found that *D. zeae* grew faster in culture at all temperatures permitting growth of the two fungi. Apparently, *D. macrospora* cannot compete with *D. zeae* when the two are growing upon the same substrate, for Hoppe (6) was unable to recover *D. macrospora* from ears of corn infected with both organisms.

As a result of studies upon various fungi growing in synthetic nutrient media, Miss Kinsel (8) announced that *Diplodia macrospora*, under the conditions of her experiment, could not utilize monosaccharides as a source of carbon, but grew readily when supplied with di- or polysaccharides. Stevens and Larsh (19), using 24 isolates of *D. macrospora* from the southeastern United States and Argentine, found that the characteristic noted by Miss Kinsel was common to all these isolates, regardless of the source of nitrogen supplied. In contrast, Miss Kinsel secured an excellent growth of *D. zeae* on media containing monosaccharides, as well as on solutions containing more complex carbohydrates. More recently, Margolin (12) has indicated that *D. macrospora* makes only a sparse growth when supplied with either sucrose or glucose, unless a needed growth substance is present in the medium. H. J. Fuller, in a paper not yet published, found that *D. macrospora* and *D. zeae* form amylase, invertase, maltase, catalase, and peroxidase in essentially the same quantities. It also has been reported that neither fungus is stimulated by thiamin (12, 15).

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[1] Material in this paper was taken from a thesis presented to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of Doctor of Philosophy in botany.

[2] Hearty thanks are due Professor Neil E. Stevens under whom this work was done for his many helpful suggestions during the course of these investigations and in the preparation of this article.

In order to obtain further information on the physiology of these fungi and, if possible, reconcile the somewhat conflicting results reported by earlier workers, further culture studies were undertaken with special reference to carbohydrate metabolism.

STUDIES ON NUTRIENT MEDIA

The basic medium used in the work described herein was the same as that employed by Stevens and Larsh, since these investigators found that *Diplodia macrospora* will grow more readily on this medium than on the more concentrated solution used by Miss Kinsel. The materials contained in this basic medium were: $MgSO_4$ 0.25 g., KH_2PO_4 0.3 g., KNO_3 2.0 g., carbohydrate 30.0 g., and distilled water to make one liter. The original source of the inoculum used in these studies was obtained from diseased ears of corn kindly furnished by P. E. Hoppe.

Since it was known that *Diplodia zeae* could utilize simple sugars, as well as the more complex carbohydrates, a check culture of *D. zeae* was always used in each lot of modified medium tested for its ability to support a mycelial growth of *D. macrospora*. The fungi were grown at room temperature in 125 ml. Pyrex flasks containing 50 ml. of medium that had been autoclaved at 15 pounds' pressure for 20 minutes. In most instances 10 flasks of each medium were inoculated.

Bits of dormant mycelium from old cultures were used as inoculum. It should be stressed that all inoculum used was brown and showed no evident signs of growth. Leonian and Lilly (11) have reported that the inoculum had no effect upon the growth of the colony of *Phycomyces blakesleeanus* in culture, but, as will be explained below, our experiments indicated that this does not hold for *Diplodia macrospora*.

Varied Sources of Inoculum. Inoculum taken from an old dormant culture of *Diplodia macrospora* invariably gave no significant growth on the basic medium containing only a simple sugar as a source of carbohydrate. A similar bit of such inoculum placed in a culture containing only whole oats produced a luxuriant mycelium. Experiment showed that a wisp of the mycelium from such a vigorously growing oat culture developed a good growth when placed in dextrose medium. It was found that hyphae from corn meal agar plates inoculated with kernels of corn infected with *D. macrospora* likewise gave rise to a sparse mycelium when placed in dextrose medium.

Removal of Trace Elements. The importance of minute quantities of heavy metals in the nutrition of fungi and the possible role such elements may play as limiting factors in the growth of fungi have been properly emphasized by Steinberg (17). Trace elements apparently are not responsible for the inability of *Diplodia macrospora* to utilize simple sugars, for, when these trace elements were removed from the nutrient solutions by the calcium carbonate method of Steinberg (16), slightly revised at his suggestion, it was found that *D. macrospora* made no growth on dextrose media so treated, but grew well on treated sucrose solutions. *D. zeae* formed abundant mycelium in solutions of both sugars whether treated or not.

Effects of Char Purification. The passage of a sucrose solution through finely ground animal char gave a complete inactivation of the sugar for growth of *Diplodia macrospora.* Purification of sucrose with Norite likewise effectively prevented development of this fungus. Other tests showed that a combination treatment of a sucrose solution with both Norite and calcium carbonate did not prevent the formation of mycelium by *D. zeae.*

Effects of Invertase Solution. A sucrose culture upon which *Diplodia macrospora* is growing gives a strong test for reducing sugars when treated with Benedict's solution. Although it is known that sucrose yields equal parts of dextrose and levulose when hydrolyzed, experiment confirmed Miss Kinsel's observation that no significant growth of *D. macrospora* resulted when the fungus was supplied with equal parts of commercial dextrose and levulose as a source of carbohydrate.

In an attempt to determine the ability of completely inverted sucrose to support growth of *Diplodia macrospora,* 10 g. of sucrose were dissolved in 330 ml. of distilled water, a 10 ml. ampoule of "Difco" invertase solution added, and the solution allowed to stand for several hours at room temperature. At the end of this time an examination of the solution by means of the polarimeter showed the sucrose to be completely inverted. After the inversion of the sugar, minerals were added and the medium flasked and autoclaved. *D. macrospora* grew well on this solution as did *D. zeae.*

The addition of an invertase solution to a glucose medium likewise made possible a heavy growth of *Diplodia macrospora,* which practically equalled the development of the fungus upon the sucrose invertase mixture. The average weight of 5 mats of *D. macrospora* grown on inverted sucrose was 470 mg.; the average of 5 mats from a dextrose-invertase solution was 450 mg. A check medium containing no other carbohydrate than that that the invertase may have carried gave only an extremely sparse growth of the two fungi.

Growth of Diplodia macrospora on Media Staled by Other Fungi. A chance observation of a flask containing *D. macrospora* on a sucrose solution that had become contaminated with an unidentified fungus showed that the growth of *D. macrospora* was much better in the contaminated flask than in the other flasks of the set. Following this lead, dextrose medium was staled by allowing *D. zeae* to grow on it for several weeks until the solution became amber or light brown. This staled medium was filtered to remove the mycelium of *D. zeae* and the filtrate was flasked and again autoclaved. *D. macrospora* made a very satisfactory growth on this staled dextrose solution. These results are similar to those reported by Allison and his coworkers in their studies on coenzyme R (1, 5).

On media containing other simple sugars, such as levulose, sorbose, galactose, rhamnose, mannite, and also glycerol, *Diploidia macrospora* made little or no significant growth, as Miss Kinsel (8) reported. *D. zeae*, however, developed satisfactorily on all of these substances. After being staled by *D. zeae* for 4 or 5 weeks, media containing these various compounds all supported a good growth of *D. macrospora*. A little staled medium added to an unstaled dextrose solution also stimulated a good development of *D. macrospora*.

Growth-promoting Substances. By using a method corresponding to that employed by Buston and Pramanik (2) to extract a growth factor from lentils, a substance was obtained from corn grains and from whole oats that exerted a definite stimulating effect upon the growth of *Diplodia macrospora*. When 2 g. of either of these grain extracts was added to a half liter of dextrose medium and the solution inoculated with *D. macrospora*, the fungus made a vigorous growth. The addition of greater quantities of the substances provided no more growth stimulus than did the smaller amount, and, in the case of the oat extract, the larger amounts inhibited mycelial growth.

Green cornstalks, stock molasses, sugar beets, and a dextrose solution staled by *Diplodia zeae*, also provided an alcohol-extractable growth substance. It is significant to note, however, that an extract derived from sucrose media staled by *D. macrospora* was not active.

A growth factor for *Diplodia macrospora* was likewise obtained by carefully removing vigorously growing mycelium of *D. macrospora* from the surface of whole-oat cultures and macerating these hyphae in a little water. The resulting liquid was then filtered, and its growth-promoting properties tested by adding a little to a dextrose medium. On such media *D. macrospora* formed a heavy growth.

Through the courtesy of Leon Leonian, a small quantity of a biotin-like substance extracted from dung was made available for testing. This material enabled *Diplodia macrospora* to form a heavy mycelium on a sucrose medium purified with Norite. Later a commercial biotin concentrate, standardized at the laboratory by the assay method of Snell, Eakin, and Williams (14), was tested. Added to a dextrose medium at the rate of 0.02 gamma per 50 ml. flask, this substance produced mats of *D. macrospora* averaging 115.5 mg. after 4 weeks. A concentration of 0.05 gamma per flask was somewhat less effective.

Ineffective Growth Substances. In an attempt to ascertain the identity of this substance required for growth of *Diplodia macrospora*, a number of known compounds were added to dextrose media. All of these compounds had been reported as growth-promoting for various fungi. Nicotinic acid and vitamin C were tested in two dilutions, 0.1 mg. and 0.5 mg. per 50 ml. flask, but induced no growth of *D. macrospora*.

Other materials tested were C.P. i-inositol, 0.2 g. per liter; vitamin B_6 kindly furnished by Merck and Co., 0.2 mg. per liter; vitamin B_1 0.2 mg. per liter; and a combination of 40 mg. of vitamin C. and 0.2 mg. of vitamin B_1 per liter. None of these materials in the concentrations tested induced any mycelial formation whatsoever by *Diplodia macrospora* in dextrose media. Media containing 0.02 gamma of biotin per flask plus 10 mg. of i-inositol gave no better growth than the flasks containing only a like quantity of biotin; nor did the addition of 0.2 mg. per flask of para-amino benzoic acid to a medium containing biotin give any increased growth.

THE PROBABLE IDENTITY OF THE GROWTH FACTOR PRODUCED BY DIPLODIA ZEAE

As noted above, media containing simple sugars as a source of carbohydrate will support a satisfactory growth of *Diplodia macrospora* only if a suitable growth factor be added. Similar media, after being staled for several weeks by *D. zeae,* will support a good growth of *D. macrospora.*

Since it was known that a commercial preparation containing biotin enabled *Diplodia macrospora* to utilize dextrose, an effort was made to determine whether the substance excreted by *D. zeae* may be identical with biotin. A concentrate obtained from dextrose media staled by *D. zeae* supported a good mycelial formation of *D. macrospora* when added to dextrose media in small amounts. For this reason the concentrate was subjected to a number of the physical and chemical treatments by which biotin, coenzyme R, and vitamin H, three growth factors now considered to be identical (4), are tested.

As a result of these tests the growth factor elaborated by *Diplodia zeae* was found to possess a number of the characteristics exhibited by biotin obtained from egg yolks by Kögl and Tönnis (9).

It is thermostable, soluble in water and alcohol, but scarcely soluble in ether, petroleum ether, or chloroform. The substance is not destroyed by refluxing with acidulated methanol, is dialyzable, adsorbed by char, and is resistant to the action of strong acids and alkalies. In addition it is completely inactivated by nitrous acid.

Two additional tests also were made upon the concentrate containing the growth factor. Following a report (3) that a constituent of raw powdered egg albumen will inactivate biotin *in vitro,* the effects of egg white were determined. A second test concerned the ability of the concentrate to stimulate the growth of *Saccharomyces cerevisiae,* an organism believed to require biotin for growth.

The whites of fresh eggs were used in testing the relative effects of raw and cooked albumen upon the growth principle in the concentrate. In the first part of this experiment the white of an egg and 10 ml. of a solution of the concentrate were added to a half liter of distilled water and the mixture autoclaved for 20 minutes. The addition of dextrose and the required minerals was made after the initial heating, and the medium was then flasked and re-autoclaved. Both *Diplodia zeae* and *D. macrospora* showed an excellent development on this medium containing the cooked white of egg.

The experiment was completed by aseptically adding the raw white of an egg to a large flask containing a half liter of dextrose medium that had been autoclaved and cooled. The flask was shaken vigorously to break up the albumen and the solution was then poured into small flasks that had been previously sterilized. *D. macrospora* made no growth in media containing the raw white of egg if no contamination appeared. In one instance where the egg was apparently already contaminated by bacteria, *D. macrospora* made a fair development after 2 or 3 weeks. The raw white of egg also reduced to a marked degree the growth of *D. zeae*.

When supplied with Domino Cube sucrose in the "B" solution used by Robbins and Schmidt (13) for the growth of *Ashbya nematospora*, a culture of *Saccharomyces cerevisiae* furnished by Leon Leonian made only a slight growth. On a similar medium, to which a little of the concentrate had been added, the yeast grew very satisfactorily.

GROWTH FACTOR PRESENT IN CERTAIN CARBOHYDRATES

The fact that *Diplodia macrospora* will grow readily on simple sugars, if supplied with suitable growth substances, suggested that the results reported by Miss Kinsel (8) and by Stevens and Larsh (19) might be traceable to impurities, present in the complex carbohydrates, removed from the monosaccharides in further processing. It also seemed reasonable to believe that very pure di- or polysaccharides also might be lacking in these substances needed for the growth of the fungus. Several tests of various carbohydrates were undertaken in an attempt to determine the facts.

Preparation of Dextrose. Since *Diplodia macrospora* was observed to grow when supplied with corn starch but did not develop on dextrose, obtained commercially by the hydrolysis of starch, it seemed desirable to study the steps in the process of the conversion of starch to dextrose. Through the courtesy of J. Paul Bishop of the Corn Products Refining Co. of Argo, Illinois, samples of hydrolyzed starch from various stages of the processing by which starch is converted to dextrose were made available. The samples tested for their ability to support growth of the two *Diplodias* were as follows: (1) starch hydrolysate neutralized with sodium carbonate; (2) the neutralized solution with fats removed by centrifuging and the centrifugal liquor filtered and passed over char once; (3) decolorized and concentrated liquor passed over char twice and ready for the crystallizer, (4) Hydrol, the second mother liquor removed from the sugar crystals by centrifuging; (5) special Hydrol, the second mother liquor specially treated to remove inorganic salts and all kinds of acids, (6) Alpha dextrose hydrate, obtained by crystallization; and (7) fatty material removed during the first centrifuging of the hydrolyzed starch solution.

These samples were evaporated to about 70 per cent dryness at the Corn Products laboratory and consequently, with the exception of the dextrose and fatty material, were used at the rate of about 40 g. per liter of medium. The results obtained are listed in the following table.

TABLE 1. *Growth record of Diplodia macrospora and D. zeae cultured for 7 weeks at room temperature*

Sample	*D. macrospora*		*D. zeae*
	No. of flasks	Av. net wt.	(1 flask)
Starch hydrolysate neutralized	9	109.4 mg.	Good
Liquor passed over char once	9	240.0	Good
Liquor ready for crystallizer	8	93.7	Good
Hydrol	9	294.4	Good
Dextrose	9	Negligible	Fair
Dextrose plus 10 g. of Hydrol per liter	9	197.2	Fair
Dextrose plus 10 g. of fatty centrifugate per liter	9	Negligible	Poor
Special Hydrol (40 g. per l.)	7	90.0	Fair
Special Hydrol (60 g. per l.)	7	97.1	Fair

Inulin. In the tests of inulin, two grades were used; Pfanstiehl C.P. and Pfanstiehl "Practical." *Diplodia macrospora* made a sparse growth when furnished with these samples of inulin in culture, averaging only 31.1 mg. per flask on the C.P. grade and 35.8 mg. on the "Practical." However, it should be noted that on another sample of C.P. inulin *D. macrospora* made a much better development. Unfortunately, no weight determinations were made of the fungus when grown on this particular sample. Inulin hydrolyzed by the addition of a little hydrochloric acid also supported a fair growth of *D. macrospora.* The best development of the fungus occurred when a little growth substance was added to the medium. *D. zeae* grew fairly well on both grades of inulin.

Maltose. When *Diplodia macrospora* was inoculated into media containing maltose the results obtained varied to a marked degree according to the grade of the sugar used. On Pfanstiehl C.P. maltose the fungus averaged only about 30 mg. per flask,[3] but, when grown in a medium containing a brown colored technical grade, a luxuriant mycelium was formed, which averaged 427.1 mg. per flask. That impurities were responsible for the stimulation of growth seems evident because passage of the sugar through finely ground animal char prevented any growth of *D. macrospora.*

Sucrose. Development also varied according to the source of the sucrose used. Sucrose samples from 5 sources were tested for their ability to support growth of *Diplodia macrospora.* These included C.P. sugar from two chemical companies, two commercial brands of cane sugar, and a sample of bulk beet sugar. The comparative results obtained are listed below.

TABLE 2. *Growth record of Diplodia macrospora and D. zeae cultured for 4 weeks at room temperature on various samples of sucrose*

Brand of sucrose	*D. macrospora*	*D. zeae*
	(Av. of 9 flasks)	(1 Flask only)
Bakers "Analyzed" C.P.	41.1 mg.	Satisfactory
Coleman & Bell C.P. Saccharose	90.0	"
C&H "Pure Cane" from 2 lb. box	48.1	"
Domino Cube	27.8	"
C&W Beet Sugar from 10 lb. bag	Very sparse	"

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[3] Average of only 3 flasks.

Coleman and Bell's C.P. sugar was commonly used as a disaccharide in our cultures, and, as a rule, permitted even more satisfactory growth of *Diplodia macrospora* than that obtained in the above comparison. The fungus consistently failed to make any significant growth on the Domino Cube sugar, unless supplied with growth factor of the biotin type. This was true also of the single sample of beet sugar tested.

DISCUSSION

The results obtained from these studies indicate that one of the fundamental physiological differences between *Diplodia zeae* and *D. macrospora* is the inability of *D. macrospora* to elaborate a growth substance required by both fungi in their metabolism. The fact that this fungus did not develop on unstaled media, containing only dextrose as a carbohydrate, but grew very well in similar media after being staled by *D. zeae,* regardless of whether or not trace elements were removed, was direct evidence that the growth of *D. macrospora* was induced by an organic substance secreted by *D. zeae.*

The occurrence of the growth factor for *Diplodia macrospora* in corn and oat grains, sugar beets, molasses, and green cornstalks indicates that this growth substance is not uncommon. The stimulating effect of invertase solution may be explained as the effect of a growth substance present as an impurity in the solution.

When grown on whole oats, *Diplodia macrospora* obtained an abundance of the required metabolite from the substrate. This was evidenced by the vigorous hyphal growth made by the fungus and by the stimulating effect that an aqueous filtrate, derived by crushing these hyphae of *D. macrospora,* exerted upon the development of the fungus in a dextrose medium. The presence of accumulated growth factor in the hyphae would account for the growth of *D. macrospora* in dextrose media when the inoculum used was bits of mycelium from vigorously growing cultures. For this reason only inoculum from dormant mycelia was used throughout the course of these investigations. The growth principle, carried over by bits of mycelium growing vigorously on agar, would seem to account for the development of *D. macrospora* obtained by Margolin when the fungus was supplied with dextrose. This appears even more probable, since a biotin-like material apparently was added to the agar on which the *D. macrospora,* used as inoculum, was grown.

The results of the various tests to which the concentrate obtained from dextrose media staled by *Diplodia zeae* was subjected indicate that in all probability biotin is the growth factor that *D. zeae* elaborates, and is a substance that both *D. zeae* and *D. macrospora* require in their metabolism. One of the most convincing pieces of evidence that *D. zeae* synthesizes biotin was furnished by the stimulating effect that the concentrate had upon the growth of *Saccharomyces cerevisiae,* a yeast believed to need biotin for growth.

As Margolin has suggested, the reason that Miss Kinsel (8) and also Stevens and Larsh (19) obtained growth of *Diplodia macrospora* only when the fungus was supplied with complex carbohydrates may be explained as the effect of a needed growth factor present as a contaminant in many di- and polysaccharides. Why the growth substance is not present in simple sugars, such as dextrose, which is obtained from a contaminated polysaccharide, is not so apparent. The tests run on the samples of processed starch that was being converted to dextrose indicated that sufficient growth substance to induce development of *D. macrospora* was present in the liquor placed in the crystallizer. Judging from the growth of the fungus, the growth factor was present also in Hydrol, the mother liquor removed by centrifuging from the dextrose crystals after the process of crystallization was completed; yet *D. macrospora* made no significant development on medium containing dextrose, unless a growth factor was supplied.

In the process of refining cane sugar a similar method for removing the molasses from the crystals by centrifuging is followed. Nevertheless, the growth of *Diplodia macrospora* indicates that an appreciable quantity of a needed growth factor may remain in association with the crystals of cane sugar. At present the only reason the writer can suggest for this fact is that the larger carbohydrate molecules may have an affinity for the growth factor, which the smaller molecules of dextrose do not possess. More study is needed before a definite explanation of this phenomenon can be given.

The very high purity of some commercial sucrose, at least insofar as the presence of growth factor is concerned, was indicated by the comparative growth of *Diplodia macrospora* obtained on these sugars and on the C.P. grades. The slight growth induced by Domino Cube sugar is apparently due to the extreme purity of the product. L. A. Wills of the American Sugar Refining Co., which produces Domino sugar, explained in private correspondence that, although ordinary commercial grades of cane sugar have a high purity of approximately 99.5 per cent, the special method of refining used in producing Domino Cube sugar gives a product that is at least 99.95 per cent pure.

The writer has no explanation of the high purity of beet sugar, as relates to the presence of growth factor, except to point out that Snell, Eakin, and Williams (14) found that sugar beet molasses in some instances contains very much less biotin than does cane sugar molasses. This may indicate that sugar-beet roots naturally contain less biotin than do cane stalks.

White has stated (21) that the source of sucrose has no significant effect upon the growth of excised tomato roots in culture, but these experiments do not warrant a similar conclusion for the growth of *Diplodia macrospora*. The high purity of the sucrose (Bakers "Analyzed" C.P.), employed by Margolin, probably accounts for the sparse growth he obtained when he attempted to culture *D. macrospora* on sucrose.

Our results emphasize that the possibility of introducing growth factors, present as impurities in the carbohydrates employed, should be borne in mind by all investigators who grow organisms in synthetic solutions. That these growth factors are more often present in complex carbohydrates than in hexose sugars is abundantly borne out by these studies. Steinberg (17) has suggested that "repetition of these tests with hydrolyzed polysaccharides would appear desirable to determine whether this effect is due to chemical structure or to the greater difficulty in purification of the polysaccharides" in those instances where it has been found that certain fungi develop more readily on polysaccharides than on simpler compounds. From the results of these studies with *Diplodia macrospora* it now appears that the more complex carbohydrates are more difficult to purify since, with the exception of one sample of dextrose, none of the monosaccharides tested was of C.P. grade. In contrast, C.P. sucrose was commonly used when a disaccharide was required.

SUMMARY

The results of this series of physiological studies on *Diplodia macrospora* and *D. zeae* having special reference to the utilization of various carbohydrates may be summarized as follows:

The source of inoculum of *D. macrospora* was found to be correlated with the ability of this fungus to grow on simple sugars supplied in the basic nutrient medium.

A substance that enabled *D. macrospora* to develop on media containing simple sugars was obtained from corn, oats, sugar beets, molasses, hyphae of *D. macrospora* from vigorously growing cultures, and a dextrose medium staled by *D. zeae*. An invertase solution and a commercial biotin concentrate were also effective in promoting growth.

The growth substance elaborated by *D. zeae* and present in a concentrate of dextrose media staled by this fungus was observed to possess many of the properties of biotin. It is believed to be biotin or a closely related compound.

Tests made on samples from various stages of the process by which starch is converted to dextrose indicated that the growth factor present in starch is not present in dextrose in significant amounts, but remains in the mother liquor removed from the dextrose after crystallization.

The growth of *D. macrospora* was definitely influenced by the source of the maltose and sucrose supplied in the culture media.

The results obtained indicate that *D. macrospora* can utilize complex carbohydrates in many instances only because they contain as an impurity a growth factor required by this fungus. This growth factor apparently is removed from simple sugars during the manufacturing processes.

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THREE BACTERIAL PLANT PATHOGENS: PHYTOMONAS CARYOPHYLLI SP. N., PHYTOMONAS ALLIICOLA SP. N., AND PHYTOMONAS MANIHOTIS (ARTHAUD-BERTHET ET BONDAR) VIEGAS

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The three bacteria described in this article have little in common except that they are plant pathogens. Two are new species, while the third has received no adequate description, although the name has appeared in literature for over 25 years.

In the following investigation, isolates of all three species were run simultaneously and the methods used were the same. To avoid repetition the methods are given in the following paragraphs. In most instances these are well known and only need be mentioned.

In measuring the bacteria the Congo red negative stain was employed, while Plimmer and Paine's modification of the Casares-Gil's stain was used for determining possession and position of flagella. The Ehrlich-Böhme method was employed for the determination of indol in tryptone broth, while strips of filter paper impregnated with lead acetate were suspended over the same medium for the determination of hydrogen sulphide production. M. P. Starr made the lipase test, using his own method, which has been published recently (17). The synthetic nitrate medium listed in the Manual of Methods (16) was employed in the nitrate studies.

In determining the substances that can be used for an energy and carbon source the following method was employed. The synthetic basal medium as recommended in the Manual of Methods (16) was used, and was tubed and autoclaved at the usual pressure and time. The carbon sources to be tested, with the exception of the salts of the organic acids, were prepared in 10 per cent aqueous solutions, sterilized by filtration, and then added to the tubes of basal media, so that a 1 per cent solution was obtained. Sodium salts of the organic acids were added directly to the basal media, making up 0.15 per cent of the solution, and autoclaved together. Ammonium lactate was used instead of the sodium salt, since this latter was not on hand. For the determination of starch hydrolysis both the starch-agar iodine method and starch broth method were used.

CARNATION PATHOGEN

Early in 1940 L. K. Jones sent the writer a number of wilted and dead carnation plants. In an accompanying letter he stated that the disease had been found in Washington State, where it was severe in one of the greenhouses, and that it was caused by a bacterium he had isolated and whose pathogenicity he had proved. In the correspondence that followed it was agreed that he should attend to the pathological phases of the disease and the writer the etiological phases—namely the description of the pathogen. Since then, Jones has published a description of the symptoms of this disease (11). In brief, the affected plants wilt, lose their color, and become dry, while the roots entirely disintegrate.

Accompanying the first specimens was also a culture of the bacterial pathogen; and later, when a great many diseased plants were received, 20 isolates were made. In most cases these were obtained from the interior of the stalks a little aboveground. Below ground, the roots were well rotted and many secondary organisms were present. These cultures were all from specimens sent from the West Coast, but it is possible that the disease is of greater distribution. In appearance it is similar to the fusarium wilt of this plant and easily might be mistaken for it. J. M. Bickerton told the writer that he had obtained at times specimens of diseased carnations that he took to be the fusarium wilt, but in which he was unable to find the fungus.

While 20 isolates were on hand at the beginning of the work only 12 were used in the various tests for determining the characteristics of the pathogen. The reduction in number was due to the death of 8 cultures that were being grown on an unfavorable medium. This medium was the ordinary beef-extract-peptone agar, and, although the cultures grew readily on it for a few days, they did not remain viable. Potato-dextrose agar was found to be a more satisfactory medium.

The pathogenicity of all the isolates was proved by inoculation into carnation plants, and after symptoms of the disease appeared the pathogens were reisolated. The carnation variety, Virginia, was used as the host plant in most experiments. Infection readily can be obtained by scraping a small mass of bacteria from a colony on agar onto a sharply pointed scalpel and, with this contaminated instrument, piercing the carnation stalk a little above the ground. Definite symptoms appear in approximately 4 weeks. Healthy carnation plants transplanted to pots that previously had disease in them or in which suspensions of the pathogen had been poured may contract the disease. Under these conditions the incubation period may be 2 months or more.

While no attempt has been made to determine the host range of the pathogen, inoculations were made on 2 plants of *Dianthus barbatus* L., and 1 plant each of *D. plumarius* L. and *D. allwoodii*, a horticultural designation of a race of hardy pinks. These were full-grown plants that happened to be in the greenhouse at the time the work was in progress. Only *D. plumarius* remained healthy.

All the isolates employed in describing the pathogen, were from single colonies, and dilution plates were made 3 to 4 times with each to insure purity. In the following tests with the 12 isolates, a uniformity of reactions existed.

Morphology. The pathogen is a short, slender rod, at times slightly curved. In 48 hour-old cultures on potato-dextrose agar at 27° C. the size of the cells was 1.84 μ (1.05 to 3.18 μ) by 0.56 μ (0.35–0.95 μ). The organism is actively motile by 1 to several flagella, frequently at both ends. It is Gram-negative.

Cultural Characteristics. Colonies, 4 to 5 days old on potato-dextrose agar are 3 to 4 mm. in diameter, circular, smooth, glistening, with edges entire. The consistency is butyrous. The color is a tan to a gray-mauve. This peculiar appearance is caused by a whitish film over the darker brown cells in the interior of the colony. Later, the film may disappear, and the colony becomes a deeper brown. On beef-extract-peptone agar the growth of the pathogen is similar to the above description, but the consistency of the colony is very firm and hard, so that it is difficult to remove portions of it. Beef-extract-peptone bouillon (pH 6.6) becomes very turbid in 24 hours at 27° C. to 36° C. A whitish sediment is formed. In litmus milk, growth is slow, the medium becomes slightly blue in 1 week, and a slight reduction of litmus begins at the bottom and advances about half way up the tube. In 6 weeks the color is a slate-blue with a dirty-white sediment. There is no clearing. In shake cultures with beef-extract, peptone agar plus 0.5 per cent dextrose used as a medium, colonies appear on the surface or only slightly below, showing that the pathogen is an aerobe. In Clara's medium (4) there is good growth but no green fluorescent pigment is produced.

The optimum temperature for growth is about 30 to 33° C. Maximum 46° and minimum 5° or less. The pathogen has an exceptionally high temperature range.

Biochemical Reactions. Growth in gelatin stabs is moderate and liquefaction begins only after 3 to 4 weeks, and proceeds very slowly. The liquefied medium is of oily consistency. Growth in tryptone broth is excellent, but indol is not formed nor is hydrogen sulphide. Asparagine, KNO_3 and $NH_4H_2PO_4$ can be utilized for nitrogen. Nitrates are reduced to nitrites, which soon disappear with the formation of ammonia and a gas. This reaction might possibly explain the destructive rot of infected tissue. Lipase is produced.

The carnation pathogen proved to be extremely saccharolytic. The following carbon sources were utilized: l-arabinose, d-xylose, rhamnose, dextrose, d-galactose, levulose (d-fructose), d-lactose, maltose and sucrose; glycerol, mannitol, and salicin, with a decrease in pH; and the sodium salts of the following acids, acetic, citric, formic, hippuric, lactic, maleic, malic, succinic, and tartaric with an increase in pH. Starch was not hydrolysed.

The above description of the bacterial pathogen and the type of disease it produces on the carnation plant, leaves little doubt that it is distinct from the disease caused by *Phytomonas woodsii* (E. F. Smith) Bergey *et al.* The latter is the only bacterial disease of carnations, as far as the writer is aware, that previously has been reported on this floral crop. *Bacterium dianthi* Arthur and Bolley now is considered a saprophyte. The description of the new carnation pathogen is so distinct in appearance and in action in media that it must be considered new. The name **Phytomonas caryophylli** n. sp. is proposed. Certain characteristics place it in the *Pseudomonas* group, as the characters of curved rods with polar flagella, the action on milk and asparagine, the use of formates and the lack of H_2S production and of starch hydrolysis.

ONION PATHOGEN

A number of bacteria have been reported in literature as causing or at least associating with rots of onion bulbs. *Bacterium allium* Griffith (9) was possibly the first to be described as isolated from rotting onion bulbs, but Griffith, who conducted the investigation, makes no claims that the species is pathogenic. The organism was evidently one of the green-fluorescent saprophytes. In 1906, Delacroix (5) isolated a bacterium from rotting onions and proved its pathogenicity. He named the pathogen *Bacillus cepivorus* n. sp., but gave an incomplete description of the organism, so that it is difficult to determine with what species he was working. Giampietro (8) claims to have found that *Bacterium coli* produces a rot of onions, and he further states that *B. cepivorus* and *Bact. coli* are identical. It is possible that both these men were working with *Erwinia carotovora* (Jones) Holland.

While the name *B. cepivorus* still appears in literature, especially from Europe, as far as the writer is aware no further work has been done on this species or the disease it produces. Numerous reports are on record that *E. carotovora* causes a soft rot of onions and several investigations from Jones (12) have proved its pathogenicity on this crop. Townsend (20) obtained a rot on sliced onion bulb with *Bacillus aroideae* when he described this species, and, later, Takomoto (18) found the same organism causing a rot of onions in Japan. *Bacillus croci* Mizusawa (13), a probable synonym of this pathogen, also has been reported on onion. Edelsztein-Kosowa (7) in Poland described a bulb rot of onion caused by a new species of bacterium, which he named *Bacillus cepae.* This pathogen is a spore former with peritrichic flagella, producing gas in carbohydrate media, and rapidly hydrolyzing starch. These characters readily differentiate it from the other pathogens.

Okabe (14) working with *Bacterium formosanum* Okabe, which the writer considers a synonym of *Phytomonas chicorii* Swingle, was able to obtain infection when onions were inoculated with this organism. Another synonym of *Phyt. chicorii* is *Phyt. endiviae* (Kotte) Clara (4). A culture of this latter bacterium was received several years ago from Kotte and recently artificial inoculations were made on onion bulbs. A definite light-brownish rot was obtained.

A number of writers have reported bacterial rots of onions without giving a name to the pathogen. The most interesting and extensive article of this type was in 1899 when Stewart (18) carefully described a rot of onion bulbs occurring in New York State, and published an excellent picture of a diseased specimen. While he was certain that the disease was bacterial he did not isolate or describe the organism. It has been thought by certain investigators that the cause of the disease reported by Stewart was *E. carotovora* but the work presented in this article throws some doubt on this point.

During the last 10 years or so, at infrequent intervals, rotting onion bulbs have been sent to the Department of Plant Pathology, Cornell University, for determination. In many instances certain inner scales of these diseased bulbs have been watersoaked and soft. In appearance the disease was not unlike a frost injury, but the rot did not extend horizontally into the adjacent scale. In early stages of the disease the bulb may appear sound on the outside, but, on being cut open, one or two of the scales may be found to be infected. In advanced stages the entire bulb may rot. Microscopic examination of even the advancing margins of the rot show they are full of bacteria.

It was thought at first, on examining such specimens, that the disease was caused by *Erwinia carotovora.* In the fall of 1936, however, attempts made to isolate *E. carotovora* from these rotting onions met with failure. In most cases, however, dirty white bacterial colonies appeared on the dilution plates and were characterized by coloring beef-extract peptone agar a deep-brown. The organism, when inoculated into onion bulbs, caused a rot, as described above. In 1939, the organism was again isolated from rotting onions. It appeared from this second instance that the bacteria were of more than casual occurrence and, since the symptoms of the disease agreed so closely with Stewart's disease, they probably were of long standing.

Just how and when infection takes place to cause this bulb rot is not known. It probably is at harvest time, or slightly before, since young growing onion leaves are but slightly susceptible to the bacterium and appear to outgrow the disease. Mature bulbs are very susceptible and may rot completely within 10 days at room temperature. No rot has been found at 6° C. upon artificially inoculated bulbs.

Inoculations were made on a number of plants with this onion organism with the following results. The bacteria are able to produce a brownish rot on carrots, which is not so soft as that caused by *Erwinia carotovora;* a black but not extensive rot on paper-white narcissus bulbs; and a slight dry rot on tulip bulbs and on iris rhizomes. Potato tubers, bulbs of *Lilium speciosum,* and cabbage stalks were not infected.

The pathogen causing this onion rot is very readily isolated from the advancing margins of the lesions by making dilution plates with either potato-dextrose agar or beef-extract-peptone agar as the medium. The latter is preferable, since a dark coloration appears around the colonies of the pathogen on this medium, and, consequently, they can be recognized more easily.

In describing this bacterium 7 isolates were used, each from a separate onion bulb and each from a single colony. Each isolate was later plated in dilution plates 3 to 4 times and its pathogenicity proved. The only difference noted among the isolates was a difference in the intensity of the brown discoloration of the media. This coloration did not have the purplish tint characteristic of the tyrosine reaction.

Morphology. The onion pathogen is a rod with rounded ends. In a 24-hour-old culture on beef-extract-peptone agar grown at 27° C., the size of cells was 2.0 µ (1.05 to 2.80 µ) by 0.9 µ (0.7 to 1.4 µ). The bacteria are motile with 1 to several polar flagella, sometimes bipolar. They are Gram-negative.

Cultural Characters. Streak cultures on beef-extract-peptone agar are moderate in growth, white at first, but, later, dirty in appearance, edges of streak wavy, consistency viscid. Agar becomes a deep brown. On potato-dextrose agar growth is more abundant, mucoid, and white to a dirty-cream in appearance. Consistency is not viscid but rather watery. Medium not brown. In beef-extract-peptone bouillon, growth appears in 24 hours or less, becoming turbid with a slight pellicle. Browning of broth begins near surface and extends downward into the tube. In litmus milk, growth is at first slow but, by 5 days, litmus is reduced and milk cleared. Medium approximately neutral. In shake cultures with beef-extract-peptone agar plus 0.5 per cent dextrose, the colonies appear only at surface. The upper quarter of medium becomes cleared and a light yellow-green in appearance. Later the medium becomes brown. In Clara's solution (4), there is a light to cloudy growth with a white sediment, but no green fluorescent pigment.

The optimum temperature for growth is about 30° C., maximum 41° C., and minimum 5° C. or less.

Biochemical Reactions. Growth in gelatin stabs is good, liquefaction begins on the second day, and in 4 weeks half of medium is liquefied and brown. Growth in tryptone broth is good, but indol is not formed nor is hydrogen sulphide. Asparagine, KNO_3 and $NH_4H_2PO_4$ can be used for the nitrogen source. Nitrates are reduced to nitrites. The organism shows excellent lipolytic action. The following carbon sources utilized: l-arabinose, d-xylose, rhamnose, dextrose, d-galactose, levulose (d-fructose), d-lactose, maltose sucrose, glycerol, mannitol, and salicin produced growth with an acid reaction. Rhamnose, however, produced a very weak growth. The salts of acetic, citric, formic, hippuric, lactic, malic, succinic, and tartaric acids produced growth with an alkaline reaction. Maleic acid was not utilized, nor was starch hydrolysed.

Taxonomy. The numerous bulb rots of onions that have been described from time to time are very similar in symptoms, and, possibly, they could be differentiated only after a comparative study of the various diseases. The bacterial pathogens causing these diseases, however, are distinctly different and are readily separated. The pathogen under consideration in this article does not agree with any of the previously described bacterial pathogens affecting onions; it appears to be a pathogen heretofore unrecognized. Bacteriologically the onion pathogen is similar to *Phytomonas solanacearum* (E. L. Smith) Bergey *et al.* It retains its virulence in culture fairly well, however, which the latter species does not do. As far as the writer is aware *Phyt. solanacearum* has not been reported on onion, and the onion pathogen does not infect potato tubers. Possibly a still more closely related species is *Phytomonas gardeniae* Burkholder and Pirone. Here again the onion pathogen produces no infection on gardenia leaves, even under very favorable circumstances. For this reason it is considered a new species, and the following name is proposed—**Phytomonas alliicola** sp. n. The pathogen probably could be placed in the *Pseudomonas* group.

CASSAVA PATHOGEN

In the summer of 1939 Karla Longrée received some cuttings of Cassava (*Manihot utilissima* Pohl.) of the variety Cambaia from A. P. Viegas of São Paulo, Brazil. The cuttings were rooted in one of the greenhouses at Cornell University and, for a time, they grew vigorously. However, after a few weeks of growth about 10 of these plants began to show signs of a wilt. The lower leaves flagged, then eventually the upper leaves and finally the whole plant died. Upon a careful examination of such plants it was discovered that the vascular system was full of bacteria that, in many cases, had broken through the cells and even on to the surface of the stem, where they occurred in sticky drops.

In correspondence with Dr. Viegas he stated that the disease was generally recognized in Brazil and was of economic importance. Bacteriologically little was known concerning the cause, and he suggested that the writer study and describe the pathogen.

In a search through literature greatly aided by A. P. Viegas it was found that 3 bacterial diseases have been described on Cassava. Bondar (1) in 1912 was the first to report one on this crop and later in 1915 (2) gave a fuller account of the trouble. The disease was found in Brazil, and the host species he gives is *Manihot palmata.* From all appearances it was this disease that was on the Cassava cutting at Cornell University and this conclusion was substantiated by A. P. Viegas. Bondar attributed the cause of the disease to a bacterium, which, in his first article, he names *Bacillus manihotus* Arthaud-Berthet,[1] but with no description. Dr. Arthaud-Berthet was collaborating with him on the problem, although his name does not appear as co-author of the article. The second article also is signed by Bondar alone but a note on page 911 of this volume states that Sr. Arthaud-Berthet's name, accidently, was omitted. Here the pathogen is called *Bacillus manihot* sp. n., and a very inadequate description is given of it. Since then the name may be found in literature as *Bacillus manihotus* Arthaud and Berthet, which is incorrect. Recently A. P. Viegas informs me that in an article in O Campo 11: No. 132; 64, 1940, which I have not seen, Bondar uses the name *Bacillus manihoti* Berthet et Bondar. Viegas (21), however, has placed the pathogen in the genus *Phytomonas* and calls it *Phytomonas manihotus* (Arth. Berth. and Bondar) n. comb. In a letter Viegas states that the "u" in the specific name is a typographical error. It should be "i."[2]

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[1] The hyphen in this name is not used consistently.

Two other diseases of Cassava caused by bacteria also are reported in literature. Miss Schwarz (15), in 1926, proved that *Phytomonas solanacearum* (E. F. S.) Bergey *et al.* was capable of attacking this plant (*Manihot utilissima* Pohl.) and causing a slime disease. Since then, others have reported finding this disease. Hansford (10), in 1938, described a leaf spot and stem disease of Cassava from Uganda. No wilt is mentioned. The pathogen *Bacterium cassavae* sp. n. belongs to the genus *Erwinia*. These two latter diseases cannot be confused with the one under consideration.

The bacteria from the affected plants in the greenhouse at Cornell University were readily isolated in either beef-extract-peptone agar or potato-dextrose agar. They conformed to Bondar's description of *Bacillus manihotus* in that they were white, grew slowly, and were aerobic. The only other character Bondar mentions is that his species was Gram-positive. The recently isolated bacteria were Gram-negative, but, as the writer has stated at other times, the Gram stain is difficult to handle, and many bacteria described at one time as Gram-positive, are now known to be Gram-negative.

In describing the present Cassava organism, 8 isolates were used. These were selected from single colonies. Dilution plates were made 3 times and the resulting isolates tested for pathogenicity. All behaved similarly both pathologically and in culture.

Morphology. The pathogen is a very slender rod. A 24-hour-old culture on beef-extract-peptone agar at 27° C. yielded the following measurements for the cells, 2.17 μ (1.4 to 2.8 μ) by 0.6 μ (0.35 to 0.93 μ). The pathogen is Gram-negative and probably nonmotile. Excellent flagella stains were obtained on the other two pathogens discussed in this article, but in only one culture of the Cassava organism could one find a very limited number of cells with a single polar flagellum. These might have been contaminants, although, in one instance, Viegas informs me he also noted motile cells.

Cultural Characters. Streaks on beef-extract-peptone agar at 27° C. show up in about 48 hours, are raised, ivory-color, smooth, shiny, with edges entire. Their consistency is watery. On potato-dextrose agar, growth is more abundant, white to hyaline, and very mucoid. Beef-extract-peptone bouillon becomes cloudy with a white granular ring. In litmus milk, growth is good and a clearing and reduction of litmus takes place in 3 to 4 days at 27° C. With the return of color to the litmus the medium is purple. In shake cultures (beef-extract-peptone agar plus .5 per cent dextrose) most of the colonies were on or at the surface. Later a few appeared deeper in the medium. There is no growth in Clara's medium.

The optimum temperature for growth is approximately 30° C., maximum 38° C. and minimum 5° C.

[2] Since this present paper was accepted for publication Drummond and Hipólito's recent investigation (6) was brought to the writer's attention. They describe the Cassava wilt pathogen and use the name *Bacterium manihotus.*

Biochemical Reactions. Growth in gelatin stabs is good. Liquefaction begins on the 4th day, and, in 2 weeks, half the medium is liquefied. Growth in tryptone broth is good. Indol is not formed but hydrogen sulphide is. The organism cannot utilize nitrates or asparagine. Ammonium salts, however, are utilized for nitrogen. There is a doubtful lipolytic action.

Since the pathogen does not grow well in synthetic media, some difficulty was experienced in determining the carbon sources it could utilize. A very weak growth with a slight acid production was obtained with dextrose, d-galactose, levulose (d-fructose), d-xylose, maltose, and sucrose. Rhamnose, l-arabinose, d-lactose, glycerol, mannitol, and salicin gave no growth. In one series a better growth was obtained with levulose when .1 per cent of sodium thio-glycolate was added than when left out. The use of this material was not followed up, however. A good growth with an alkaline reaction was obtained with the salts of the organic acids, acetic, citric, malic, maleic and succinic. The salts of formic, hippuric, lactic, and tartaric were not utilized. Starch was not hydrolysed.

For the present this organism had best be placed in the genus *Phytomonas* and the following name used, *Phytomonas manihotis* (Art.-Berth. et Bondar) Viegas. Steps are being undertaken, however, to drop this genus and allocate its members to other genera. Where this species then would be placed has not been determined. It seems to be one of those intermediate types having relationships with the genus *Pseudomonas* and that small group of Gram-negative vascular parasites, as *Phytomonas albilineans* (Ashby) Magrou, *Phytomonas stewartii* (Erw. Smith) Bergey *et al.* and *Phytomonas tardicrescens* (McCulloch) Burkholder. Further work on its relationships to other bacteria is needed.

SUMMARY

A description is given of three bacterial pathogens. Two are new species, **Phytomonas caryophylli** sp. n., which causes a wilt and root rot of carnations, and **Phytomonas alliicola** sp. n., which causes a bulb rot of onions. The third description is of *Phytomonas manihotis* (Arthaud-Berthet et Bondar) Viegas, a bacterium causing a wilt of Cassava and of which no adequate description has been given hitherto.

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LEAF-SPOT DISEASE OF CULTIVATED SALSIFY

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(Accepted for publication May 19, 1941)

In August, 1933, the writer found on Staten Island, New York, an apparently undescribed leaf spot of cultivated salsify (*Tragopogon porrifolius* L.), caused by a variety of *Stemphylium botryosum* Wallr. The lesions were confined to the foliage and varied in size from barely perceptible spots to those 3–4 mm. in diameter. Symptoms somewhat similar to these are described by Aderhold[1] for a leaf spot on *Scorzonera hispanica* L. (black salsify) in Germany and by Archer[2] for a leaf spotting of *T. porrifolius* in West Virginia. In both of these cases the disease is caused by *Sporodesmium scorzonerae* Aderh. Morphological studies show that this fungus is entirely different from the one here described.

SUSCEPT RANGE

The only known suscepts are *Tragopogon porrifolius* and *T. pratensis* L. (goat's beard), a fairly common weed in the northeastern United States. Although the disease has not been found on the latter plant under natural conditions, successful inoculations of young, potted plants were made with spore suspensions of the pathogen. The symptoms are similar to those on *T. porrifolius*. Both young and nearly-mature plants of *Scorzonera hispanica* were similarly but unsuccessfully inoculated. In addition, plants of this species were grown in the field in the same rows with cultivated salsify plants. Although the latter eventually became severely blighted, the black salsify plants remained healthy.

DISTRIBUTION AND ECONOMIC IMPORTANCE

The disease seems to be rather limited in distribution, partly because the suscept is not widely grown. A letter was sent out in 1938 requesting diseased material or cultures of the pathogen. Replies were received from pathologists in 19 vegetable-growing states. No reports of any salsify leaf-spot diseases were received, although a plant pathologist in Ohio wrote that he had "seen salsify in one or two instances which was affected with other things than white rust but the trouble never seemed important enough to cause much concern." These replies indicate that salsify is grown on a rather limited commercial scale in 10 States and not at all in 4. The extent of salsify culture in 5 States was not ascertained.

Diseased leaves have been collected each year since 1933 in every commercial planting on Staten Island. The only other collection of which there is a record is that made in 1938 in a small planting near Rochester,

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[1] Aderhold, R. Ueber eine bisher nicht beobachtete Krankheit der Schwarzwurzeln. Arb. Biol. Abt. für Land- und Forst. 3: 439–440. 1903.

[2] Archer, W. A. Plant Diseases in West Virginia in 1928. Bur. Pl. Ind., U. S. Dept. Agr. Plant Dis. Rptr., Suppl. 72: 346. 1929.

New York, by A. G. Newhall. The question as to why this disease is so localized is as yet unanswered.

The total area annually devoted to salsify on Staten Island amounts to less than 2 acres and has a value of approximately $1,000. The annual loss from this disease averages approximately 5 per cent of the crop, attributable largely to reduced size and quality of roots. On 2 occasions during the past 6 years, the disease became so severe following prolonged warm, rainy periods that some plants lost all of their foliage. Under enphytotic conditions, which ordinarily prevail, this loss is not more than 30 per cent. Occasionally, soft-rot organisms invade the roots through the severely blighted leaves, the resulting root decay contributing substantially to the economic loss.

SYMPTOMS

The initial symptoms of the disease appears as light-brown lesions on the tips of the older leaves. These ordinarily appear after August 1, when the plants are nearly half-matured. Soon thereafter, minute, light-brown, necrotic spots, surrounded by light-green areas develop on the leaf blades (Fig. 1, A). Later, these lesions enlarge until they are from 3 to 4 mm. in diameter and become cinnamon-brown with ashen-gray centers (Fig. 1, B). A reddish-brown exudate, composed mainly of the natural milky juice of the plant, sometimes can be found on the smaller lesions on both upper and lower leaf surfaces. The larger spots often coalesce and involve con-

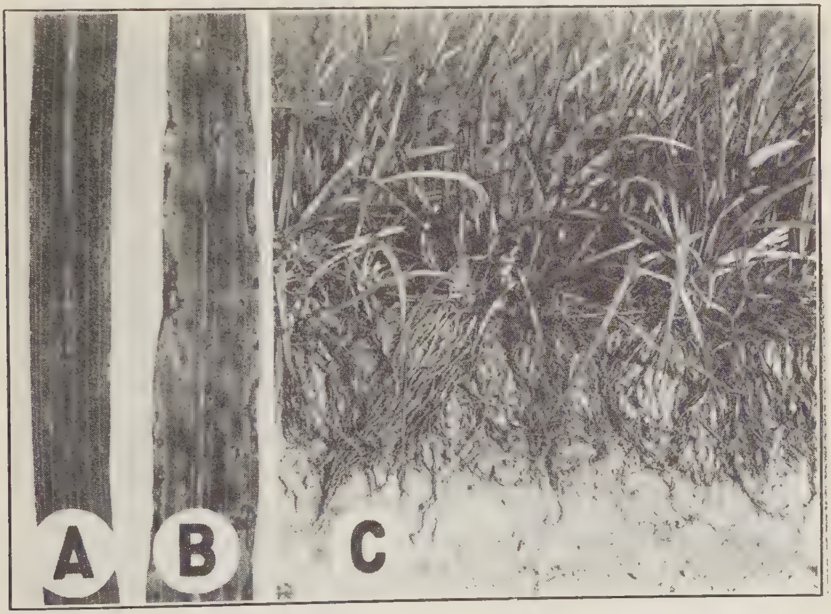

FIG. 1. Symptoms of stemphylium leaf-spot disease of *Tragopogon porrifolius* (cultivated salsify). A, incipient and B, advanced stages of spotting. C. Death of foliage resulting from early-season infections.

siderable areas of the leaves. Severely-affected leaves gradually turn yellow and wither (Fig. 1, C).

THE PATHOGEN

Morphology. The fungus grows readily on potato-dextrose agar and forms in both Petri-dish and test-tube cultures a spreading, cottony growth marked by faintly-zonate bands of conidiophores and conidia. The mycelium is at first light-brown and, finally, a color approaching olive-ocher.[3] The relatively hyaline mycelium ramifies throughout infected leaf tissue, transverse growth in the leaf occurring prior to any considerable amount of lateral growth.

The conidiophores, which are borne in fascicles on the superficial mycelium, are light-brown, occasionally branched, 4.0–4.5 μ wide by 25–180 μ long, and swollen at the apex to a diameter of from 6 to 8 μ (Fig. 2, D, E). The lower two-thirds of the apical swelling is darker than the upper portion and is thickened by an inner wall. Each conidiophore bears a single apical spore. The conidiophore may continue growth (proliferate) through the swollen apex and the scar left by the fallen spore, with a second spore produced terminally. This phenomenon results in the formation of so-called nodular segments, although not more than two of these have been observed on single conidiophores produced either on lesions or in culture.

The spores are light-brown and minutely verrucose, measure 17–56 × 8–26 μ, are ovoid-oblong to subangular, have one to several longitudinal walls and 1–3 transverse walls, and are without appendages or beaks (Fig. 2, C). The spores are slightly constricted at the transverse walls. A basal scar is visible. Occasionally, on germination in culture, the spores give rise directly to conidiophores that proliferate and produce acrogenous spores.

Aderhold's[4] description of the spores of *Sporodesmium scorzonerae* (inverted club-shape and long-drawn-out, measuring without the appendage 50–75 × 13.5–16.5 μ, and with 10–12 cross walls and 1–2 longitudinal walls) leaves no doubt as to the dissimilarity of the two pathogens (Fig. 2, A). Archer (2) identified the causal agent of a leaf spot in West Virginia as morphologically similar to if not identical with *S. scorzonerae.* The spores measured 18–54 × 7.5–8 μ, being somewhat smaller than those described by Aderhold. An examination of spores found on lesions on the diseased material collected by Archer (No. 4147, Path. and Mycol. Coll., Bur. of Pl. Ind., U. S. Dept. of Agr.) lends further proof of the separate identity of the fungus found on Staten Island (Fig. 2, B).

Taxonomy. Cultures of the causal fungus were sent to S. P. Wiltshire of the Imperial Mycological Institute, Kew, England, who identified it as a species of *Stemphylium* (subgenus *Eustemphylium*). According to him[5] it differs from the type species (*Stemphylium botryosum* Wallr.) only

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[3] Ridgway, R. Color standards and color nomenclature. 43 pp., 53 colored plates. (Washington) 1912.

[4] See footnote 1.

[5] Personal letter dated April 16, 1940.

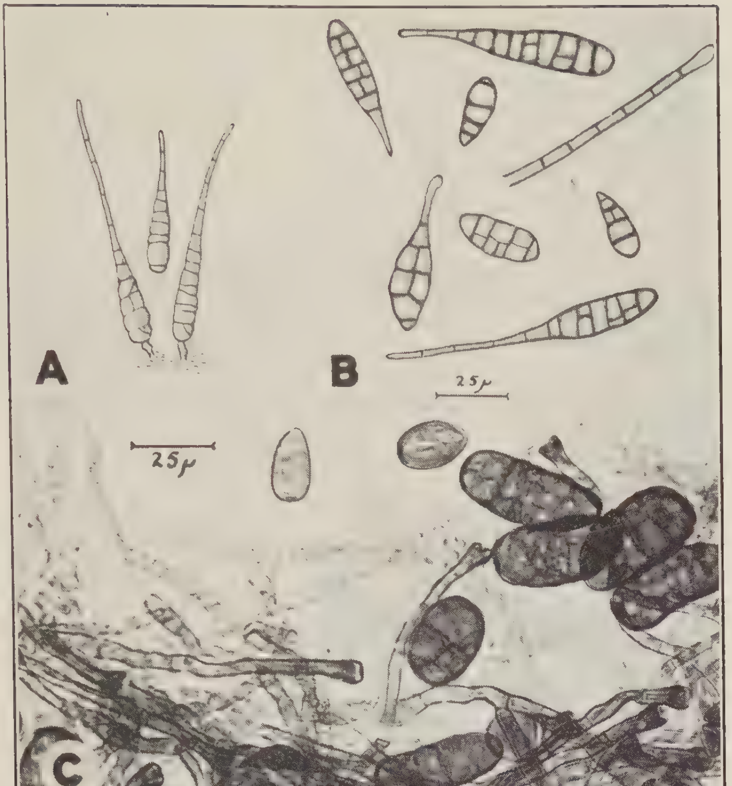

FIG. 2. A. A copy of a drawing by Aderhold of spores of *Sporodesmium scorzonerae* taken from *Scorzonera hispanica* (magnification unknown). B. Camera-lucida drawing of spores of *S. scorzonerae* found on *Tragopogon porrifolius* by Archer. C, D, E. Photomicrographs of spores and sporophores of *Stemphylium botryosum* var. *tragopogoni* from *T. porrifolius*.

in that the spores are longer than is usual for this species (24–39 × 19–31 μ). He was unable to obtain perithecia on exposure of the cultures to light, thus confirming the results of similar experiments performed by the writer. Dr. Wiltshire writes further that in the absence of the perithecial stage "perhaps the least objectionable course would be to regard it as a strain or variety of *S. botryosum* for the time being." Wiltshire[6] has stated that if his conception of the specific limits is correct, the synonyms of *S. botryosum* will probably be comparatively numerous. For example, he considers this name to be synonymous with *Macrosporium sarcinula* Berk. (*M. parasiticum* Thüm.), which has a wide range of suscepts. Under these conditions, it would seem unwise to create a new species on the basis of spore measurements alone, without first making numerous cross inoculations. Therefore, it is proposed that the name *Stemphylium botryosum* Wallr. *Tragopogoni* n. var. be assigned provisionally to this pathogen on *Tragopogon porrifolius*.

Stemphylium botryosum Wallr. **tragopogoni** n. var. Mycelial mat spreading, cottony, faintly zonate, at first light-brown, finally olive-ocher; hyphae septate, branching, light-brown, 3.0–3.5 μ thick; conidiophores arising in fascicles from superficial mycelium, light brown, occasionally branched, swollen at the apex to a diameter of 6–8 μ, proliferating through the apex and fallen spore scar, 4.0–5.5 μ wide by 25–180 μ long; conidia borne acrogenously, light brown, minutely verrucose, 17–56 × 8–26 μ, ovoid-oblong to subangular, 1 to several longitudinal walls and 1–3 transverse walls, constricted at the transverse walls, basal scar visible.

Type material (infected leaves of *Tragopogon porrifolius* and dried agar cultures) has been deposited in the herbarium of the Department of Plant Pathology, Cornell University (Accession No. 25439).

PATHOGENICITY

The pathogenicity of the fungus has been demonstrated repeatedly by inoculating the leaves of potted salsify plants with spore suspensions from single-spore and mass cultures. These plants were then maintained under bell glasses at high humidity and at temperatures of 21–27° C. The first symptoms, consisting of small, water-soaked, irregular areas, became evident within 24 to 36 hours after inoculation. The bell glasses then were removed, and within another 24 hours small, brown spots appeared on these hydrotic areas. If the bell glasses were not removed, conidia were produced on the infected areas within 72 hours after inoculation. Lesions also developed if blocks of agar bearing the mycelium, alone, were placed on the leaves.

EFFECT OF TEMPERATURE ON GROWTH AND SPORE PRODUCTION

The pathogen grows most rapidly and fruits most abundantly at moderately high temperatures. The fungus was grown on 2 per cent potato-dextrose agar (pH 6.5) in triplicated Petri dishes at temperatures ranging from 6° C. to 33° C. The average colonial diameters in millimeters after 10 days were as follows: 6°, no growth; 9°, 17; 12°, 23; 15°, 30; 18°, 44; 21°, 55; 24°, 80; 27°, 52; 30°, 38; 33°, no growth. Spore production was correlated closely with growth: no spores forming either below 15° C. or above 30° C. and the maximum number between 21° and 27° C. Variations in chromogenesis in the media were marked throughout the above temperature series: dark green at 9° C., through yellowish-brown at 24° C. to pink at 30° C.

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[6] Wiltshire, S. P. The original and modern conceptions of Stemphylium. Trans. British Mycol. Soc. 21: 211–239. 1938.

SEED TRANSMISSION

In order to determine if salsify seed is susceptible to invasion by the pathogen under artificial conditions, and, therefore, a possible agent of transmission, flowers in full bloom in the greenhouse were atomized with spore suspensions of the fungus. The flowers were then enclosed for 36 hours in cellophane bags containing moistened cotton. The bags were then removed and the seed allowed to mature in the flowers. Following harvest the seed was stored in a dry place for 2 months. At the expiration of this period, 100 of these seeds were given individually an instantaneous dip in 95 per cent alcohol, rinsed immediately in water and treated in calcium hypochlorite solution (1:14) for 5 minutes. Following this treatment they were planted on potato-dextrose agar. Cultures of the pathogen were obtained from 80 per cent of the seed. Four months after harvest another lot of this seed was disinfested as above and planted on agar. None yielded cultures of the pathogen. Germination tests made immediately after harvest showed that only 5 per cent of the inoculated seeds were viable, while tests made again at the end of 4 months revealed that by this time none of the seed was viable. Similar tests with seed from noninoculated and unbagged flowers showed that 70 per cent of the seed germinated immediately after harvest and 65 per cent at the end of 4 months after harvest. These results indicate that under certain artificial conditions, not conducive to normal development of the seed, the fungus can penetrate the seed coat and remain alive for relatively short periods of time.

There is no evidence that the pathogen is carried in or on commercial seed despite the demonstrated susceptibility of the seed under abnormal conditions. Fresh seed was obtained from 12 seed companies and from growers on whose farms the disease is enphytotic. Equal representative samples from each lot were either planted in agar or sown in steam-sterilized soil. In some instances the seed was sown without treatment; in others it was disinfested either in calcium hypochlorite solution (1:14) for 5 minutes or $HgCl_2$ solution (1:1000) for 5 minutes followed by two rinses in sterile water. The pathogen was never recovered from the culture media or isolated from the seedlings.

In other experiments, seed from growers' stocks was sown on farms which had not grown salsify for 10 or more years and which were at least ½ mile from the nearest commercial planting. The resulting plants remained healthy throughout the season. If, however, old, blighted leaves were raked into the soil around some of the young seedlings, blight developed on these plants by the latter part of August. When this seed was sown on farms that had grown salsify year after year, blight appeared on the resulting plants in due course.

It would seem that transmission of the pathogen in or on commercial seed, if it ever occurs, is comparatively unimportant in the life history of the fungus. The fact that the disease appears to be confined almost entirely to Staten Island is a further indication of the minor role played by the seed in the dissemination of the pathogen.

SOIL-BORNE INOCULUM

It has been established through experiment that the fungus is capable of overwintering for at least a year on blighted leaves in the field. Severely-diseased salsify leaves were wrapped in cheesecloth and buried 3 in. below the surface of the soil on Staten Island in late October. Part of the leaves were dug up in July of the following year. One portion was strewn over the leaves of five 6-month-old, potted salsify plants, while a second portion was sterilized in an autoclave before being scattered over a similar series of plants. All plants, including noncontaminated checks, were sprayed with water and placed in an incubation chamber under near optimum conditions for infection. Symptoms of blight developed only on those plants contaminated with nonsterilized, blighted leaves. No stromatic tissue was observed in or on the overwintered leaves although viable spores were found immediately after the leaves were taken from the ground. The remainder of the buried leaves was left in the ground for an additional 12 months. Inoculation experiments, similar to those detailed above, demonstrated that the pathogen was no longer capable of causing infection. Spores could not be found. It is likely that, under some conditions, the longevity of the pathogen in the soil exceeds one year. In any event, the amount and disposition of soil-borne inoculum is believed to be an important factor in conditioning the prevalence and severity of the disease.

CONTROL

Control experiments have been directed mainly toward finding a protective fungicide for the foliage. As a result it has been found that the disease can be controlled readily by spraying with Bordeaux mixture if applications are begun before the older leaves bend over to the ground. Preliminary laboratory and field tests revealed that both 325-mesh sulphur dust and wettable-sulphur sprays were relatively ineffective in preventing spore germination. Copper-lime dust (20–80) was highly toxic to the spores in laboratory tests but did not give satisfactory control in the field because the dew forms in droplets on the waxy-leaf surfaces instead of in a continuous film. Bordeaux mixture (4–4–50) and Cuprocide 54 (Rohm and Haas) ($1\frac{3}{4}$ lb. to 50 gal. water) gave excellent coverage when amended with suitable spreading agents. The best spreaders (1:400) were Pyrolene M.P. (Standard Agricultural Chemicals) and Ultrawet (Atlantic Refining Co.). Others tested were Penetrol (Kay Fries Chemicals), Stantex spreader soap (Standard Agricultural Chemicals), Grasselli sticker and spreader, Grasselli IN–438 (sodium oleyl sulphate), and Santomerse (Monsanto Chemical Co.).

In a series of experiments in 1938 Bordeaux mixture and Cuprocide 54 were applied with a knapsack sprayer, commencing on July 7 when the plants were from 6 to 10 in. tall, and continuing at weekly or biweekly intervals until Oct. 5, 1 week before harvest.[7] Treatments were replicated twice on each of 3 farms with individual plots measuring approximately 10 sq. ft. Data were taken at harvest on the average number of spots per leaf, including both healthy and diseased leaves, on 10 plants chosen at random in each plot. There was no significant difference in this respect between plots receiving weekly applications of either Bordeaux or Cuprocide 54 and those sprayed biweekly. Plants sprayed biweekly with Bordeaux had fewer lesions (1.3 per leaf) than either those sprayed with Cuprocide 54 (6.1) or the nonsprayed checks (11.6). On the basis of root weights correlated with the number of roots required to make a bunch, Bordeaux- and Cuprocide-treated plots yielded approximately 200 more bunches to the acre than did the checks. There was no appreciable yield difference between these two treatments.

SUMMARY

An apparently undescribed leaf spot of cultivated salsify, *Tragopogon porrifolius,* caused by a variety of *Stemphylium botryosum,* occurs commonly in commercial plantings on Staten Island, New York. *T. pratensis* (goat's beard), a common weed, inoculated with the causal fungus, proved susceptible.

The most prominent symptoms are small, light-brown, necrotic spots that cause affected leaves to turn yellow and wither.

The pathogen is morphologically unlike *Sporodesmium scorzonerae,* previously reported on *T. porrifolius* and *Scorzonera hispanica,* and is described herein as *Stemphylium botryosum* Wallr. *tragopogoni,* n. var.

Under optimum conditions of temperature and humidity, inoculation of the suscept results in the formation of lesions within 48 to 60 hours. The optimum temperature for growth and spore production in culture is between 21 and 27° C.

There is some experimental evidence that commercial seed is not an important factor in the overwintering and dissemination of the pathogen.

Soil-borne inoculum, provided by old, blighted leaves from the previous crop, is believed largely responsible for the enphytotic occurrence of the disease.

The disease can be controlled readily through biweekly applications of Bordeaux mixture or Cuprocide 54, provided the spraying program is begun before the older leaves bend over to the ground and provided a suitable spreader is used.

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[7] The common practice on Staten Island is to harvest most of the crop before the first killing frost. The roots are sold in the New York City market in bunches of from 10 to 20 each.

VARIABILITY IN REACTION OF WHEAT DIFFERENTIAL VARIETIES TO PHYSIOLOGIC RACES OF TILLETIA LEVIS AND T. TRITICI[1]

H. A. Rodenhiser and C. S. Holton[2]

(Accepted for publication May 25, 1941)

INTRODUCTION

Experiments previously reported (2) gave evidence that environmental factors may affect the response of certain varieties to some races of the bunt fungi. In those experiments race 2 of *Tilletia levis* Kühn produced 77 per cent infection on Turkey wheat (C.I. 6175) at Pullman, Washington, as contrasted with 16 per cent infection when the same lot of inoculated seed was sown at Kearneysville, West Virginia. Similar differences were obtained with races L–1 and L–4. Five other races of *T. levis,* however, showed no notable differences at the two locations in their reaction on Turkey. The fact that Hybrid 128 (C.I. 4512) gave the same reaction to all of the races tested both at Kearneysville and at Pullman suggested that the environmental effect was expressed only in the reaction of certain varieties to certain races. Obviously, this has a direct bearing on race classification in the bunt fungi. It seemed desirable, therefore, to investigate the problem in detail, particularly with reference to the identification and classification of physiologic races. This paper presents the results of tests that were made to determine the range of variability in the reaction of spring wheat differential varieties to certain races of *T. levis* and *T. tritici* (Bjerk.) Wint.

MATERIALS AND METHODS

Lots of each of the 3 spring wheat differential varieties, Ulka (C.I. 11478), Marquis (C.I. 3641), and Canus (C.I. 11637), were inoculated individually with chlamydospores of each of 7 physiologic races of *Tilletia levis* and of 7 physiologic races of *T. tritici* and then grown at 5 experiment stations in 1937 and 7 stations in 1938. Seed of each variety was grown at Pullman, Washington, in 1936 and used as the stock for succeeding years' tests. Before inoculation it was treated with formaldehyde according to the standard dip method, thoroughly washed with water to remove all traces of formaldehyde, and allowed to dry. Inoculum of each race used was increased on its differential host each year at Pullman, and then stored during the winter at 10° C. Powdered inoculum of each of the races was applied at the rate of 0.5 g. to 100 g. of seed. To insure a uniform spore load sufficient seed of each variety was inoculated in one lot then divided for tests at the several stations. In 1937 and 1938 the inoculated seed was spring-sown at the rate of 5 g. to the row in duplicate 5-foot rows at Arlington, Virginia; Bozeman, Montana; Aberdeen, Idaho; and Pullman; and at the same rate in fall sowings at Pendleton, Oregon. Tests at Logan, Utah, and at St. Paul, Minnesota, were added to the list of spring seedings in 1938. The smut percentages shown were based on counts of the total number of healthy and infected heads in each row. Fractional percentages of 0.5 or more were recorded as 1 per cent; those below this fraction were disregarded.

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[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Idaho, Minnesota, Montana, Oregon, Utah and Washington Agricultural Experiment Stations.

[2] Pathologist and Associate Pathologist, respectively, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

EXPERIMENTAL RESULTS

Races of Tilletia levis

Data on the reaction of Ulka, Marquis, and Canus to 7 races of *Tilletia levis* are recorded in table 1. It will be noted that Ulka, used as a check variety, was highly susceptible to each of the races at all of the stations in both seasons. Furthermore, there were no outsanding differences in the percentages of infection on this variety in different localities nor in the 2 seasons in which the tests were made at any one station. A possible exception is Pullman, Washington, where the average for all races was 74 per cent in 1937 as compared with 97 per cent in 1938. The virulence of all races at this station was, however, uniformly less in 1937 than in 1938; and it seems probable that this difference resulted from the less favorable conditions for infection in 1937.

In contrast to the uniform reaction of Ulka, Marquis was extremely variable in the tests in different localities and to some extent in different years, at least at one station. Thus, in 1937, the average percentage of infection on Marquis for all races, was 7 at Arlington, Virginia, 15 at Pullman, Washington, 39 at Bozeman, Montana, and 57 at Aberdeen, Idaho. Likewise, in 1938, there was a wide range in the general averages of infection in different localities. The average infection with all races was only 6 per cent at St. Paul and 7 per cent at Arlington, but at Aberdeen the average was above 50 per cent. The reaction of Marquis to L–3 illustrates the extreme variability that may occur in the response of this variety to an individual race. In 1937 there was 5 per cent infection at Arlington as compared with 12 per cent at Pullman, 46 per cent at Bozeman, and 64 per cent at Aberdeen. Thus, according to the system of race classification adopted by the writers (2), the above reactions would be classified as resistant, intermediate, or susceptible, depending upon the locality in which the test was made. Where Marquis was tested with the 7 races of *Tilletia levis* for 2 years at the same stations the results were constant at certain stations and variable at others. The differences in infection percentages at Arlington and Aberdeen between 1937 and 1938 were slight. At Pullman, the average infection was 15 per cent in 1937 and 33 per cent in 1938. It seems likely, however, that the difference was due, as in the case of Ulka, to less favorable conditions for infection in 1937. At Bozeman, the seasonal response was very marked. The average infection on Ulka was 87 per cent in both years. On Marquis, however, the average percentage of infection

TABLE 1.—*Infection of Ulka, Marquis and Canus by seven physiologic races of Tilletia levis in different localities in 1937 and 1938*

Race No.	Smut infection at													
	Arlington, Va.		Aberdeen, Ida.		Pullman, Wash.		Bozeman, Mont.		Logan, Utah		St. Paul, Minn.		Pendleton,[a] Oreg.	
	1937	1938	1937	1938	1937	1938	1937	1938	1937	1938	1937	1938	1937	1938
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
							Ulka							
L–1	82	86	97	98	85	97	93	90	......	95	......	76	86	93
L–2	76	88	97	98	74	98	89	88	......	94	......	68	89	93
L–3	83	91	97	98	77	99	78	89	......	95	......	90	95	94
L–4	86	88	96	98	73	97	90	89	......	94	......	65	93	83
L–5	94	91	96	95	71	94	89	91	......	97	......	66	95	88
L–7	95	86	97	81	65	97	84	80	......	97	......	93	60	98
L–8	92	90	98	92	71	99	82	83	......	92	......	74	94	97
Av. all races	87	89	97	94	74	97	87	87	......	95	......	76	87	92
							Marquis							
L–1	10	4	38	53	18	28	30	11	......	15	......	2	30	71
L–2	9	15	81	64	38	48	57	32	......	21	......	14	61	82
L–3	5	15	64	53	12	40	46	20	......	50	......	3	56	79
L–4	11	5	37	51	12	30	37	16	......	30	......	7	51	76
L–5	7	17	70	48	8	21	47	17	......	36	......	3	37	74
L–7	1	7	34	37	9	18	14	14	......	25	......	3	32	67
L–8	5	13	72	48	10	46	40	15	......	30	......	8	55	83
Av. all races	7	11	57	51	15	33	39	18	......	30	......	6	46	76
							Canus							
L–1	0	1	1	4	2	2	3	3	......	0	......	1	4	26
L–2	0	0	1	3	1	0	6	0	......	0	......	1	13	27
L–3	21	30	59	58	29	46	64	32	......	44	......	9	68	81
L–4	0	0	1	0	0	0	2	0	......	0	......	1	6	12
L–5	0	5	8	23	1	8	14	7	......	17	......	1	10	28
L–7	13	26	67	59	25	30	69	29	......	31	......	13	55	84
L–8	27	42	66	60	49	32	77	33	......	44	......	10	65	87
Av. all races	9	15	29	30	15	17	33	15	......	19	......	5	32	49

[a] Fall-sown.

for all races was 39 in 1937 and only 18 in 1938, and the percentage of infection with some of the races differed widely in the two seasons. On the basis of the uniform infection of Ulka, it seems evident that the variability in the infection of Marquis in the two seasons was due to the effect of environment on the host rather than on the bunt fungi. Furthermore, the environmental effect seemed more marked with certain races than with others. For example, L–7 produced 14 per cent bunt on Marquis in both years, while L–8 produced 40 per cent in 1937 and only 18 per cent in 1938.

Unlike Marquis, which was highly variable in its reaction to all of the races, Canus was constant in its reaction to certain races in different localities. As shown in table 1, the resistance of Canus to L–1, L–2, and L–4 was maintained in all localities both years. Like Marquis, however, it was variable in its reaction to those races to which it is susceptible. For example, on the basis of the 1938 results with L–3, Canus would be classed as resistant at St. Paul, intermediate at Bozeman and Arlington, and susceptible at Pullman and Aberdeen. Canus was relatively constant in its seasonal reaction at Arlington, Aberdeen, and Pullman. However, in Canus as in Marquis, the average percentages of infection at Bozeman were much higher in 1937 than in 1938.

Races of Tilletia tritici

The tests with races of *Tilletia tritici* appear to corroborate the results obtained with races of *T. levis*. As shown in table 2, Ulka was, in general,

TABLE 2.—*Infection of Ulka, Marquis, and Canus wheats by seven physiologic races of Tilletia tritici in different localities in 1937 and 1938*

Race No.	Smut infection at													
	Arlington, Va.		Aberdeen, Ida.		Pullman, Wash.		Bozeman, Mont.		Logan, Utah		St. Paul, Minn.		Pendleton, Oreg.	
	1937	1938	1937	1938	1937	1938	1937	1938	1937	1938	1937	1938	1937	1938
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
							Ulka							
T–3	82	72	97	84	74	98	94	89	......	93	......	68	89	86
T–4	93	81	99	93	81	98	93	91	......	93	......	91	87	93
T–5	87	74	98	97	78	96	89	89	......	84	......	83	85	96
T–7	87	76	96	97	62	99	91	90	......	96	......	63	89	93
T–8	72	70	96	97	56	88	91	87	......	92	......	50	85	92
T–9	93	79	93	98	76	96	73	79	......	88	......	63	91	95
T–10	6	4	13	13	28	11	3	13	......	6	......	2	5	65
Av. all races	74	65	85	83	65	84	76	77	......	79	......	60	76	89
							Marquis							
T–3	12	7	54	42	19	22	54	17	......	22	......	4	25	85
T–4	15	6	84	59	25	30	47	16	......	27	......	3	40	76
T–5	5	4	53	49	29	38	44	19	......	37	......	2	42	80
T–7	9	5	73	55	11	31	56	21	......	27	......	4	35	68
T–8	6	3	70	52	20	30	59	14	......	14	......	2	45	84
T–9	4	1	11	10	1	7	9	4	......	5	......	1	15	58
T–10	3	5	41	44	7	33	28	10	......	25	......	2	33	78
Av. all races	8	4	55	44	16	27	42	14	......	22	......	3	34	76
							Canus							
T–3	17	14	41	47	39	32	63	20	......	29	......	14	27	74
T–4	0	0	2	0	0	0	4	0	......	0	......	1	3	17
T–5	16	11	62	47	59	32	58	25	......	35	......	8	43	74
T–7	1	1	15	15	4	8	16	4	......	7	......	3	4	52
T–8	19	24	60	48	14	40	60	18	......	30	......	11	52	77
T–9	1	0	4	1	8	0	3	0	......	1	......	0	5	20
T–10	0	6	1	0	1	0	1	0	......	0	......	0	3	32
Av. all races	8	8	26	23	18	16	29	10	......	15	......	5	20	49

consistent in its reaction to races of *T. tritici* in all of the tests. On the other hand, the reactions of Marquis and Canus were rather variable; and as in the case of *T. levis,* the variability is expressed in the reaction with certain races and not with others. Both Marquis and Canus were fairly consistent in their reaction to the individual races in the 2 years at Arlington, Aberdeen, and Pullman, but at Bozeman the response of these varieties to certain races was not the same both years.

Some of the effects of different races of *T. tritici* are noteworthy. Under certain conditions, upon nearing maturity, Ulka tends to become purplish-brown in the glumes and on part of the neck. When infected with some races, particularly T–10, this pigmentation is greatly intensified. At Aberdeen, Idaho, only 13 per cent of the culms from seed inoculated with T–10 produced smut balls in the head. However, many other heads were observed with the intensified pigmentation and with small shrunken grains, which indicated that the smut mycelium was in the plant but had failed to develop chlamydospores in the ovaries. A similar condition was noted at Bozeman. It thus seems evident that Ulka is more susceptible to race T–10 than is indicated by the percentages of infection shown in table 2.

As previously stated, Canus is resistant to 3 races of *Tilletia levis,* and the resistance was maintained when tested in different localities or years. Similar results were obtained with the *T. tritici* races, T–4, T–9, and T–10 on Canus and with T–9 on Marquis. However, in the races of *T. tritici* to which the 2 varieties are susceptible, the variability was very marked, and at Bozeman results differed in the 2 years. At the other stations the behavior in the 2 years was fairly constant. At Bozeman the average infection for all races was 42 per cent in 1937 as compared with 14 per cent in 1938. Again, it appears that this variability was due to the effect of environment on the host rather than on the fungi, as tests with Ulka indicate that the chlamydospores of all races had an equally high degree of viability in both seasons. Canus was affected in a similar manner, as evidenced by the results involving the races to which that variety is susceptible.

The percentages of infection recorded in tables 1 and 2 are based upon the average of counts in duplicate rows. It seemed desirable, therefore, to check the results with more replications. The three varieties were tested in 1939 with L–2, L–7, T–4, and T–5 in five replications in spring plantings at Arlington, Bozeman, Aberdeen, Pullman, and Logan. These data, recorded in table 3, clearly corroborate the results of previous tests. Ulka was uniformly susceptible to each of the 4 races; but both Marquis and Canus were again variable in their response to certain races and could be classed as resistant, intermediate, or susceptible, depending upon the locality in which the test was made. It will be noted that Canus again maintained a high degree of resistance to races L–2 and T–4 at all stations.

There is evidence that certain spring wheat varieties are less susceptible to bunt when planted in the spring than in the fall. In this connection, when Hope (C.I. 8178) was spring-sown at Bozeman in 1936 and 1937, it

TABLE 3.—*Average infection of Ulka, Marquis, and Canus wheats inoculated with two races of Tilletia levis and two of T. tritici, and tested in five replications in different localities in 1939*

Race No.	Variety	Smut infection at				
		Arlington, Va.	Aberdeen, Ida.	Pullman, Wash.	Bozeman, Mont.	Logan, Utah
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
L–2	Ulka	93.9	94.0	93.3[a]	67.0	87.3
L–7		91.4	92.2	93.4	65.5	87.1
T–4		92.1	92.4	95.7	67.7	93.1
T–5		89.4	91.7	94.4	72.1	85.1
L–2	Marquis	9.7	67.1	77.2[a]	26.2	22.9
L–7		1.5	30.2	41.5	17.0	9.0
T–4		4.7	41.7	62.4	21.1	7.2
T–5		8.4	49.6	56.3	23.0	10.0
L–2	Canus	0.5	0.0	0.0[a]	0.0	0.0
L–7		8.0	44.2	38.0	25.7	13.6
T–4		0.0	1.1	0.2	0.0	0.0
T–5		4.9	36.0	46.0	23.2	14.4

[a] Average of 4 replications.

was highly resistant to each of the races listed in tables 1 and 2. However, when fall-sown at Pendleton in 1938, this variety was completely susceptible to all of the races of *Tilletia levis* as well as to races T–8, T–9, and T–10 of *T. tritici* and was intermediate in reaction to T–3, T–4, T–5 and T–6. These results are in agreement with those reported by Heald and Gaines (1) and Smith (3, 4). In the experiment here reported the wheats were fall-sown at Pendleton, Oregon, in tests otherwise comparable to those from spring seeding at the other stations. The percentages of infection on Ulka at Pendleton with all races except T–10 (tables 1 and 2), were approximately the same as at the spring-wheat stations. There were some noteworthy differences, however, in the smut reactions on Marquis and on Canus. On these two varieties the average percentages of infection in 1938 were higher at Pendleton than in the other tests made from spring seedings. The percentages at Pendleton were lower in 1937 than in 1938, but approximately equal to or higher than those obtained at 3 of the 4 other stations. The important differences between reaction under fall and spring-seeding conditions are expressed with certain races. It has previously been noted that Canus maintained its resistance to several races at all of the spring wheat stations. When tested under fall-sowing conditions at Pendleton, however, the resistance was broken down to some extent, particularly in 1938. The average percentage infection of Canus with L–2 at all the spring-wheat stations in 1938 was 0.7 as compared with 27 at Pendleton. With L–4, the corresponding differences were 0.2 and 12 per cent and with T–7, 6.3 and 52 per cent. The breakdown in resistance of Marquis to race T–9 in 1938 was also marked, the average percentage infection for the spring-wheat stations being 4.7 in contrast to 58 for the tests at Pendleton.

The explanation for the differential response of varieties to individual races of the bunt fungi under different environmental conditions is largely speculative. Results reported by Woolman (5) and corroborated in preliminary tests by the writers, indicate that the bunt fungi enter the epidermis of seedlings of both susceptible and resistant varieties with equal facility. It appears then that the extent to which these fungi may set up parasitic relationships with the host depends upon the physiologic condition of the protoplasm. This protoplasmic resistance or susceptibility is doubtless governed by definite genetic factors, but the expression of these factors may be modified by environment. Thus it would be expected that certain varieties would differ in response to certain races when grown under different environmental conditions. Inasmuch as Ulka was uniform in its response to each of the races when spring-sown in the different localities, it would appear that the variability in response of Marquis and Canus when grown under different environmental conditions was due primarily to the effect of environment on the host rather than on the fungi. Somewhat less probable explanations are that the variability may have resulted from "varietal screening" of the races of fungi or from differential effect of environment on different biotypes.

SUMMARY AND CONCLUSIONS

Bunt experiments at 7 experiment stations demonstrate that a difference in environmental conditions may affect the response of some spring-wheat varieties to certain races of *Tilletia levis* and *T. tritici.* The effect is not evident, however, in the reaction of some varieties with certain other races. The Ulka variety was constant in its reaction at all stations when inoculated with the races to which it is susceptible. Marquis, on the other hand, was so variable in its response to certain races at different stations, that, according to the race classification used by the writers, this variety would be classed as resistant, intermediate, or susceptible to those races, depending upon the location of the tests. In contrast, this variety maintained a high degree of resistance to race T–9 at all stations where the wheat was sown in the spring. Canus was variable in its response in the different localities to the races to which that variety is susceptible but was constant in reaction to the races to which it is resistant.

The most probable explanation for the differential response of varieties to individual races of the bunt fungi under different environmental conditions is that the expression of genetic factors for protoplasmic resistance in the host is modified differently in the different varieties by environment. That is, the host rather than the fungi showed the primary effect of environment.

The data obtained tend to increase the complexity of race identification and of testing for smut resistance in spring wheats. At two stations relatively high percentages of infection were obtained on the susceptible check variety, Ulka; and some differences seem evident in the pathogenicity of certain races on Marquis and Canus. At three other stations the separation of the races was more distinct, while at Aberdeen the environmental conditions were nearly optimum for infection and for demonstrating the potentialities of individual races. It is evident from the data obtained that tests for varietal resistance should not be confined to a single area as it is possible for a race to be relatively innocuous under what appears to be optimum conditions for infection and then to assume major importance in another environment.

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A LABORATORY APPARATUS FOR STUDYING SETTLING RATE AND FRACTIONATION OF DUSTS

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INTRODUCTION

At the outset of research on dusting it became necessary to design a laboratory apparatus to study settling rate and fractionation of dust clouds. Apparatus previously described[3] (1, 2, 3, 4, 5, 6) were not entirely suited for such studies. Accordingly, an apparatus was designed embodying the following features: a simple method of introducing dust into a settling tower, a "magazine" (charge tube) from which practically instantaneous and complete discharge of dust could be obtained by an air blast, and an exposure chamber at the base of the settling tower by which slides or leaves could be serially exposed to the dust cloud. This apparatus has been found to give consistent results.

DESCRIPTION OF THE APPARATUS

The Settling Tower

The tower was made of cellulose acetate film supported by 3 rings cut from plywood and suitably braced (Figs. 1, 2). The top was sealed by cardboard. The tower is 6 feet high and 10 inches in diameter, the cross sectional area being 78.54 sq. in. A 1-in. projection of the film was left at the bottom, below the last ring, to fit into the top of the exposure chamber. This tower is similar in many respects to that developed at Ohio State University.[3, 4]

When in use the tower is grounded[4] by wetting the film with a 10 per cent calcium chloride solution, placing a strip of tinfoil around the base, and attaching a grounded copper wire to the tinfoil. This eliminates trouble from static electricity.

Exposure Chamber for Making Serial Exposures

This can be made any size, as long as its cross sectional area is larger than that of the tower. The one constructed (Figs. 1, 2) is 18 in. square and 6.5 in. high. A hole is cut in the top just large enough to allow the 1-in. projection of the film to fit snugly.

One in. from the top of the chamber a slot is cut to allow for a shutter; this shutter fits into a slot at the back to hold it in place.

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[2] Assistant Entomologist, Dept. of Entomology, Conn. Agr. Exp. Sta.

[3] Anonymous. Application of insecticides to leaves in controlled quantity. Ohio State University, Columbus, Ohio. (Mimeographed paper.)

[4] This tower is described anonymously in a mimeographed paper. This paper was secured through the courtesy of Dr. H. A. Waters, Ohio State University Research Foundation, Columbus, Ohio.

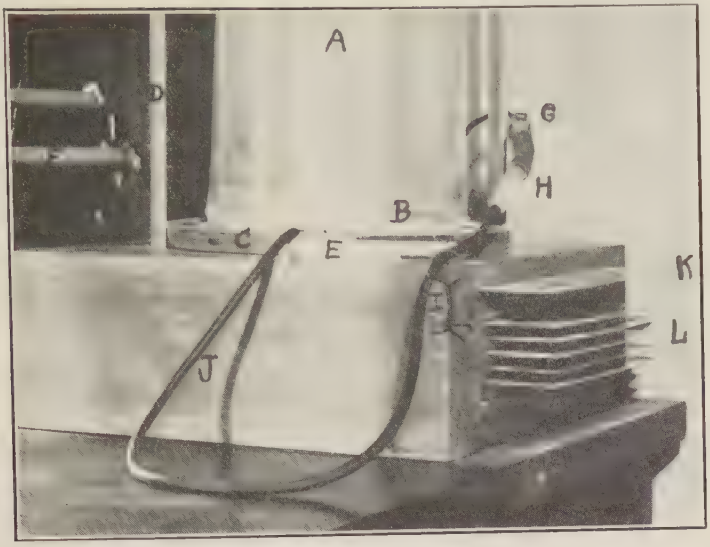

FIG. 1. General view of complete apparatus. A–F, Basal part of tower: A. Cellulose acetate film; B. Tin foil strip; C. Basal plywood ring; D. Plywood strip for support; E. Clamp for holding copper tube inserted in tower; F. Fine copper wire from tin foil to ground on water pipes. G–J. Mechanism for introducing dust into the tower: G. Push button air valve; H. Magazine (charge tube) for holding dust; I. Pinch clamp; J. Rubber tube leading from outlet end of magazine to copper tube inserted in the tower. K–L. Exposure chamber: K. Shutter; L. Aluminum slide holders.

In the front of the chamber, 1.25 in. below the slot for the shutter, a rectangular area, 12.75 × 3 in., is cut out to allow for the insertion of a drawer (Fig. 2). When in place, this drawer fits into grooves at the sides

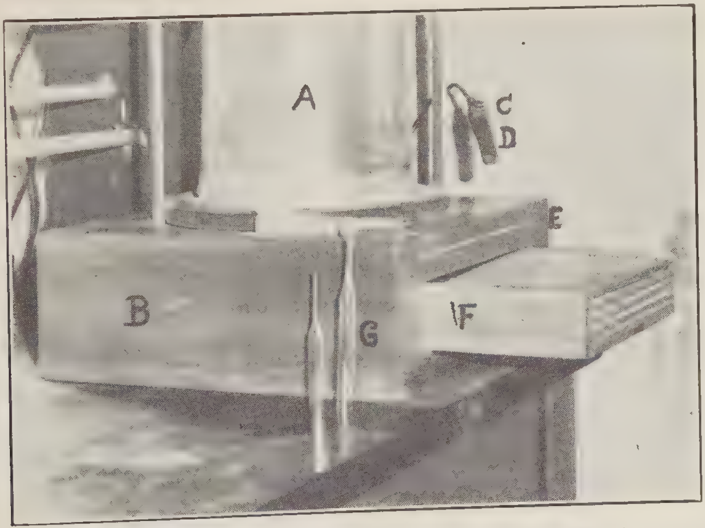

FIG. 2. Detailed view showing certain features. A, Basal part of tower. B. Exposure chamber. C. Push button air valve. D. Large rubber tube from air valve, open end fits over large end of the dust magazine. E. Slot for shutter. F. Drawer fitting into exposure chamber, showing slots for slide holders. G. Dust magazines.

and back of the chamber. The drawer has 5 slots (Figs. 1, 2), each slot being 0.25 in. deep and separated by partitions 0.25 in. thick (Fig. 2).

Sheet aluminum, $\frac{1}{16}$ in. thick, is cut to fit in these slots (Fig. 1) and used to hold the slides or leaves to be dusted. The area of the sheets directly under the center of the tower is marked so that the slides or leaves can be centered.

By this arrangement, 5 exposures can be made at different times to the cloud of dust.

If small potted plants are to be dusted, all that is necessary is to remove the drawer, place the plant in the bottom of the chamber, and close the opening with a piece of cardboard.

Dust Magazine

The dust magazine (charge tube), for instantaneous and uniform discharge of the dust placed in it, should be of certain construction. Two of these are shown in figure 2. For work with relatively large charges, 1 to 3 grams, the tube should be 7 in. long—for 4 inches of its length its inside diameter is $\frac{1}{2}$ in., after which it tapers at one end for a distance of approximately 1 in. until its inside diameter is $\frac{1}{8}$ in. for the last 2 inches. For smaller charges of dust smaller magazines can be used but they should be of the same general construction. The location of this dust magazine is described below.

Various other types of charge tubes were tested and found unsatisfactory. Those that are just large glass tubing with a cork containing a smaller tube at each end, the small tubing at one end connecting with the air supply and the one at the other end being the dust outlet, have been found particularly unsatisfactory for two reasons: not all the dust is discharged and some of it tends to pack at the flat surface of the cork at each end. Those tubes that have the outlet end similar to the "magazine" described above but in which the air enters through a tube in a cork at the other end have been found unsatisfactory also, as the dust tends to swirl back and pack at the corked end. For practically instantaneous and complete discharge of dust from the magazine the tube for the entrance of air should be of the same diameter as the magazine.

Method of Introducing Dust Into the Apparatus

Various methods of introducing dust into the tower were tried. The following method was found to give consistent results.

A copper tube, $\frac{1}{8}$ in. inside diameter, was inserted into the tower at the base through a hole in the cellulose acetate film and held in place by a clothes-pin clamp (Fig. 1). The tube was bent at a right angle in the center of the tower, the vertical extension of the tube being 2 inches. A rubber tube was attached to the end outside the tower (Fig. 1) and connected with the outlet end of the dust magazine. A pinch cock was placed in the line just below the connection. A large rubber tube (Fig. 2), of the same diameter as the magazine, was fitted over the large (loading) end of the magazine, the other end of the tube being connected to the valve[5] in the air line. This valve operates by a push button.

The entire apparatus, ready for use, is shown in figure 1.

The loading of the magazine is simple. The pinch cock is closed, the rubber tube removed from the large end, the dust poured into the tube, the rubber tube replaced, and the pinch cock opened.

To eject the dust from the magazine into the tower all that is necessary is to push the button in the air valve. All the dust in the magazine is forced out practically instantaneously (less than $\frac{1}{4}$ sec.) by the air blast (15 lb. pressure), and is ejected into the tower in less than one-half second. At 15 lb. pressure most dusts are blown to a height of 5 feet in the tower.

OPERATION AND USES OF THE APPARATUS

The settling rate of a dust is determined as follows: Tarred lantern slides, 4″ × 3.25″, are placed on each of the 5 slide holders. A known charge of dust is placed in the dust magazine. If no settling of the aggregates and large particles is desired before exposures are made the top shutter is pulled out, thus exposing the first slide. The dust is then ejected into the tower by opening the air valve for 1 second, timed with a stop watch or metronome. The copper tube is then withdrawn from the center of the tower to avoid interference with the dust cloud. At the 5th second the first slide holder is pulled out, thus exposing the second slide; at the 15th second the second slide holder is pulled out, exposing the 3rd slide; the rest of the slides are handled in similar fashion for the desired time periods. They are then weighed to determine the amount of dust deposited at the various intervals. The time intervals can be varied to suit conditions or the operator.

If it is desired to allow aggregates and large particles to settle before exposures are made, the top shutter is left in while the dust is being ejected and is then pulled out at the desired time, exposing the first slide. Exposures are then made at the desired times as described above.

When fractionation of the component parts of a dust mixture is studied, the procedure is the same as above, except that the deposits are analyzed after weighing to determine the percentage of the various ingredients present at the different time intervals. If more or less of one ingredient is present than calculated at the different exposure periods, fractionation of the mixture has occurred.

Several other types of studies can be made with this same apparatus. These include the deposition of uniform and known amounts of dust on leaves or slides for toxicity studies, deposition of dust on wet and dry surfaces for tenacity studies, and the effect of materials such as oil on settling rate.

In using the apparatus it has been found that the deposit of dust is very uniform over the exposed area of the slide holder, except for a ring about ¼ in. wide at the edge where the deposit is heavier during the first few seconds of settling.

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[5] Schrader "Blow Gun" valve, one-fourth inch, No. 7184.

The apparatus should be used in a dimly lighted room, away from sources of light and heat, to obviate convection currents in the tower.

CONSISTENCY OF DATA

The consistency of data obtained with the apparatus is shown by data (Table 1) on the amount of deposit of dust mixtures in a definite time period.

TABLE 1. *Consistency of data obtained with the apparatus, using various dust mixtures*

Dust mixtures[a]	Exposure time[b]	Deposit (13 sq. in.)			
		Rep. 1	Rep. 2	Rep. 3	Average
	Sec.	*G.*	*G.*	*G.*	*G.*
Copper-talc (7–93)	30	.0416	.0421	.0428	.0422
Copper-flour-talc (7–10–83)	30	.0405	.0350	.0380	.0378
Copper-clay (7–93)	30	.0340	.0320	.0350	.0337
Copper-flour-clay (7–10–83)	30	.0340	.0340	.0370	.0350

[a] 1 gram charge of dust used.
[b] 5 second settling period allowed before exposures were made.

A test was made also on the consistency of data obtained for exposure for the first 5 seconds of settling, no presettling being allowed (Table 2). Various diluents were used in this work.

TABLE 2. *Consistency of data obtained with the apparatus, exposing slides for a short period and using various diluents*

Material[a]	Exposure time[b]	Deposit (13 sq. in.)			
		Rep. 1	Rep. 2	Rep. 3	Average
	Sec.	*G.*	*G.*	*G.*	*G.*
Aplite	5	.0490	.0483	.0430	.0468
Cherokee clay	5	.0555	.0565	.0575	.0565
Pyrax ABB	5	.0295	.0310	.0300	.0301
Volclay Bentonite 200M	5	.0383	.0416	.0358	.0386

[a] 1 gram charge of dust used.
[b] No presettling allowed.

The data for the replicates agree surprisingly well in view of the fact that there are two main sources of error in the use of the apparatus: (a) opening the shutter and (b) pulling out the slide holders.

SUMMARY

An apparatus is described for use in studying the settling rate and fractionation of dusts. The features of this apparatus are a settling tower of simple design, an exposure chamber for making serial exposures of slides

or leaves to the dust cloud in the settling tower, a dust magazine (charge tube) from which almost instantaneous and complete discharge can be obtained by an air blast, and a simple mechanism for introducing known charges of dust into the settling tower.

Consistent results have been obtained with this apparatus.

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3. FISHER, R. A. A dust delivery tube for laboratory experiments with contact insecticides. U.S.D.A. Bur. of Ent. & Plt. Quar. ET-151. 1939.
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CEPHALOSPORIUM LEAF SPOT OF DIEFFENBACHIA

M. B. LINN

(Accepted for publication May 19, 1941)

INTRODUCTION

Throughout the past 2 years, a leaf spot of *Dieffenbachia picta* Schott (family—Araceae), caused by a species of *Cephalosporium*, was prevalent in greenhouses on Staten Island, New York. The spots were sufficiently large and numerous to make unsalable approximately 400 out of a total of 3,000 plants. A comparison of the symptoms of this disease with those of the bacterial disease of *Dieffenbachia* described by McCulloch and Pirone[1] shows the resemblance between them to be slight. In addition, congo-red tests[2] reveal that bacteria, ordinarily, are not present in the lesions, although colonies of saprophytic bacteria sometimes develop in cultures from lesions and leaf tissue.

SYMPTOMS

The initial infections appear on the young, convolute leaves as tiny, reddish-brown, circular to elongated lesions (Fig. 1, A). As these leaves unroll and expand, the lesions increase in diameter to 6 to 8 mm. At this stage they are mostly circular in outline and rather sharply delimited by a dark-brown border (Fig. 1, B). The central portion is grayish. Elongated lesions are found occasionally on the petioles and midribs, and even on the stems, under conditions extremely favorable for the development of the fungus. Coalescence of the lesions, and yellowing and death of entire leaves are, however, rarely encountered under greenhouse conditions.

PATHOGENICITY

A species of *Cephalosporium* has been isolated repeatedly from the lesions, and infection has been obtained both on young, convolute leaves and on older, fully-expanded ones by atomizing them with spore suspensions from single-spore cultures of the isolates. Symptoms appear on the inoculated plants within 3 to 5 days if they are kept at relatively high humidities and at temperatures of from 21° to 27° C.

Infection may occur through both ventral and dorsal leaf surfaces and more readily through wounds, *e.g.*, needle punctures, than through nonwounded surfaces. Although both young and old leaves are susceptible to infection through wounds, only the convolute or the recently-unrolled leaves are susceptible if the surfaces are not wounded. The majority of the lesions on fully-expanded, mature leaves are initiated when the leaves are in the convolute stage.

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[1] McCulloch, Lucia, and P. P. Pirone. Bacterial leaf spot of Dieffenbachia. Phytopath. 29: 956–962. 1939.

[2] Burkholder, W. H. A bacterial leaf spot of geranium. Phytopath. 27: 554–560. 1937.

Observations in the Staten Island greenhouses indicated that the common mealy bug (*Pseudococcus citri* Risso), with which these plants often are infested, was responsible for the infection courts (feeding injuries), since the favorite breeding and feeding areas of these pests on *Dieffenbachias* are under the leaf sheaths surrounding the immature leaves. This hypothesis was tested by the following experiment. Several mealy bugs were collected from English ivy plants, which are not susceptible to the disease, and placed in the uppermost leaf sheaths of ten *Dieffenbachia* plants on which the tips of the new leaves were just beginning to show. At the end of 4 weeks the new leaves were atomized with a spore suspension of the pathogen. A series of noninfested (wound-free) plants were inoculated similarly. A third series of infested and noninfested but noninoculated plants were maintained as controls. Within 5 days numerous lesions had developed on the new leaves with a preponderant number in a more or less linear arrangement above the infested leaf sheaths. Those on the noninfested (and wound-free) plants were considerably less in number and distributed more or less

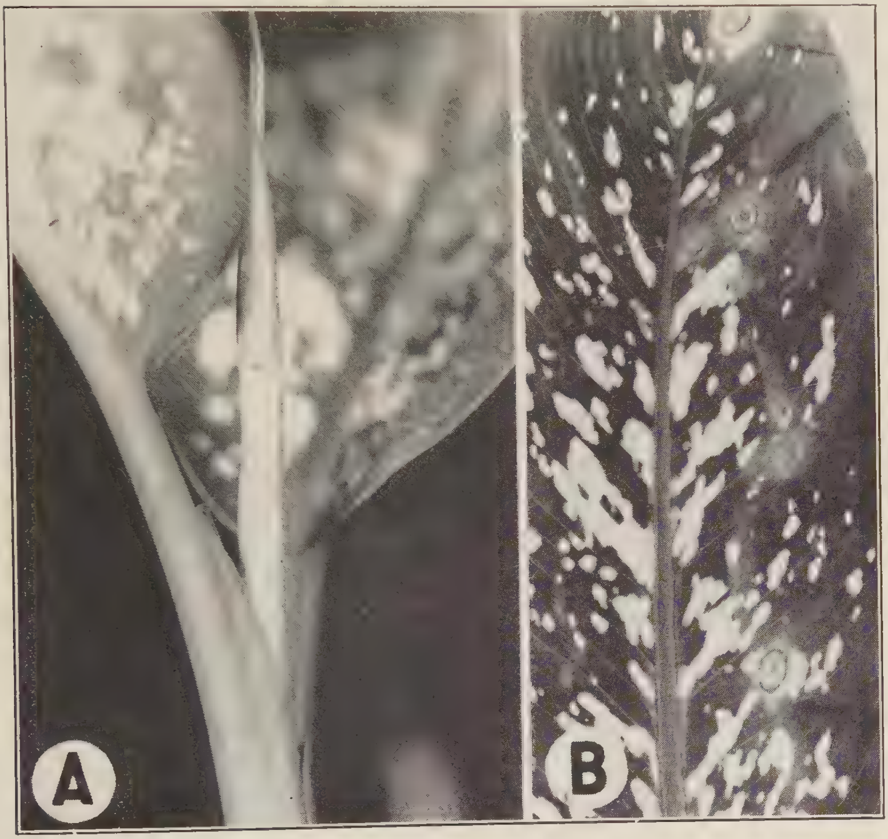

FIG. 1. A. Initial symptoms of *Cephalosporium* leaf spot on young, convolute leaves of *Dieffenbachia picta.* B. Advanced stages of the disease on a mature leaf.

uniformly over the entire, exposed leaf surfaces. The controls remained healthy.

Cross-inoculation experiments with the *Cephalosporium* from *Dieffenbachia* and with *Cephalosporium cinnamomeum*[3] from *Nephthytis afzelii* Schott, using 20 plants each of *Dieffenbachia* and of *Nephthytis*, demonstrated that the two pathogens are specific in their suscept range and not cross-inoculable.

CULTURAL CHARACTERISTICS OF THE PATHOGEN

On 2 per cent potato-dextrose agar the fungus forms a whitish growth becoming cartridge-buff[4] with age, never cinnamon-buff, as in *C. cinnamomeum.* The mycelial mat is appressed and pulverulent. The branched, septate mycelium gives rise to lateral, upright, simple to 1–2 branched, continuous to septate conidiophores, that, for the most part, are straight and not tapering. Decumbent conidiophores are not produced. This is in contrast to the situation in *C. cinnamomeum,* where the spores from such conidiophores often impart a watery appearance to the surface of the culture. The acrogenous conidia are abstricted singly and collect in grayish-white, slimy, spherical heads. The conidia are ovoid to short-cylindric and have from none to 2, rarely 3 or 4, septa. Chlamydospores have not been observed.

TAXONOMY

The *Cephalosporium* on *Dieffenbachia* differs from *C. cinnamomeum* in (a) suscept range, (b) color in culture, (c) the size of the conidia, (d) the consistency of the mycelial mat, and (e) the shape of the conidiophore. It differs from other species described in the literature principally in suscept range and in color in culture. While there is reason to believe that this fungus may be related closely to some of these species, especially *C. cinnamomeum,* there is no way at present, short of a monographic study of the group, to determine the extent and nature of that relationship. Therefore, it is proposed that the pathogen on *Dieffenbachia picta* be given the name **Cephalosporium dieffenbachiae** sp. nov.

TECHNICAL DESCRIPTION OF THE PATHOGEN

Cephalosporium dieffenbachiae, sp. nov. Mycelial mat creeping, appressed and pulverulent, white, becoming cartridge-buff with age; hyphae septate, branching, 2–4 μ thick; conidiophores arising laterally from the mycelium, aerial, simple to 1–2 branched, continuous to septate, mostly straight, 1.5–2.0 × 4.0–47 μ; conidia borne acrogenously, hyaline, ovoid to short-cylindric, 1.5–2.5 × 3.0–12.5 μ, none to 2, rarely 3 or 4, septa, collecting in grayish-white, slimy, spherical heads measuring 5–12 μ in diameter. On leaves and stems of *Dieffenbachia picta* Schott.

Type material including infected leaves of *D. picta* and dried agar cultures of the pathogen have been deposited in the Herbarium of the Department of Plant Pathology of Cornell University (No. 29413).

[3] Linn, M. B. Cephalosporium leaf spot of two aroids. Phytopath. **30**: 968–972. 1940.

[4] Ridgway, R. Color standards and color nomenclature. 43 pp., 53 col. plates (Washington). 1912.

CONTROL

Although experiments on control have not been attempted, enough is known of the factor influencing the spread and development of the pathogen to warrant some suggestions for control. These include segregation of infected plants, avoidance of syringing, and maintenance of temperatures and humidities as low as is consistent with good cultural practice. Control of mealybugs is highly desirable, but is considerably more difficult of attainment than the other practices.

SUMMARY

The symptoms of a leaf-spot disease of *Dieffenbachia picta,* caused by a species of *Cephalosporium,* are described. The initial infections occur on the young, convolute leaves. The pathogen enters more readily through wounds than through nonwounded surfaces. Most of the wounds are believed to be made by mealybugs feeding under the sheaths surrounding the youngest leaves.

Under experimental conditions, this pathogen and *C. cinnamomeum,* previously reported on *Nephthytis afzelii,* were not cross-inoculable on *Nephthytis* and *Dieffenbachia.*

The cultural and morphological characteristics of the fungus are described in detail.

The name **Cephalosporium dieffenbachiae** sp. nov. is proposed for the pathogen on *D. picta.*

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RELATION OF TUBE LAYERS TO AGE IN SPOROPHORES OF FOMES IGNIARIUS ON ASPEN

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(Accepted for publication May 31, 1941)

It has been assumed quite generally that the age of sporophores of the genus *Fomes* can be determined by counting the number of stratified tube layers in the hymenophore.[2] The writers have found no published studies, however, that demonstrate the accuracy of this assumption. Buller[3] (pages 106–107) cites Faull as having ascertained that *Fomes fomentarius* (L.) Gill., ". . . develops each new tube layer in the autumn." This suggests the probable production of 1 tube layer annually in this species of *Fomes*. White[4] states that, "The sporophores of *Fomes applanatus* are perennial and so a new tube layer is formed during the growing season of each year of its life." He found that the tubes commenced to form by the end of April and their growth was almost complete by the end of July. White, however, does not state whether his observations on tube formation extended beyond a single growing season. Buller[3] indicates (pages 116 and 126) that *Fomes igniarius* (L.) Gill. produces a new annual layer during the first part of the summer, but that it is not known whether each layer of tubes in a fruit body of *F. igniarius* represents the whole of the year's growth. Since a reliable index to the age of perennial sporophores is useful in studying the decay of trees, a project was begun in 1935 to determine the relation of hymenophore stratification to age, using sporophores of *Fomes igniarius* growing on aspen, *Populus tremuloides* Michx.

METHODS

At the Charles Lathrop Pack Demonstration Forest, Warrensburg, New York, 6 aspen trees with 20 sporophores of *Fomes igniarius* on their trunks were selected for study. On August 18, 1935, the sporophores were cut from the trees, care being taken to remove all of the sporophore tissue. The location of each sporophore scar was marked on the trunks with white paint. Each spring, summer, and autumn thereafter the 6 trees were examined and records were made of the season and year when new sporophores appeared on the exposed branch scars. On September 6, 1940, 5 years after the project was started, the new sporophores were removed from the trees and the number of tube layers in each hymenophore was counted.

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[1] This project was initiated by the junior author while he was a graduate assistant in the Department of Forest Botany and Pathology at the New York State College of Forestry, Syracuse, New York. Subsequent field observations and records were made by the senior writer.

[2] In this paper, the term "hymenophore" is used to designate that portion of a sporophore consisting of the tube layers, *i.e.*, the trama and the hymenium.

[3] Buller, A. H. R. Researches on fungi. Vol. 2. (London). 1922.

[4] White, J. H. On the biology of *Fomes applanatus* (Pers.) Wallr. Trans. Roy. Canadian Institute. Pages 133–174. 1919.

RESULTS

When the trees were examined on April 4, 1936, the first spring after removal of the original sporophores, a thick layer of newly formed mycelium covered each of the 20 areas previously occupied by the sporophores. At this time 18 of the mycelial formations had shallow pores on their exposed surface, and by summer another of these formations had developed a distinct pore surface; the single remaining mycelial mass never formed pores. In July of the same year a weft of mycelium was found just emerging from a branch scar that previously had not borne a sporophore. By autumn this weft had taken the form of a young sporophore with a pore surface. This sporophore was included in the study; other sporophores that developed subsequently during the 5 years' investigation were not recorded. Thus, there was a total of 20 sporophores of definitely known age.

When the 20 sporophores were removed at the end of the 5-year period, it was found that in 17 of them the age, by summer seasons, corresponded exactly with the number of tube layers (Table 1). In sporophores 3, 14, and 15 the number of tube layers was less than the age in seasons. These 3 sporophores remained small and were almost enclosed by callus at the time they were removed from the trees (Fig. 1, B).

TABLE 1.—*Twenty Fomes igniarius sporophores of known age upon aspen for which the number of tube layers are recorded*

Tree No.	Sporophore No.	Sporophore age in years	No. of tube layers	Sporophore size in cm.[a]	Remarks
1	1	5	5	6.4 × 3.8 × 1.0	
	2	5	5	5.2 × 2.3 × 1.1	
	3	1	0		Callused over 7–3–1937.
2	4	1½	2	4.5 × 4.5 × 0.7	Tree dead, cut down, sporophore removed 7–3–1937.
	5	1½	2	4.0 × 4.5 × 0.7	Same as above.
3	6	2	3[b]	....	Tree dead, left standing and sporophore removed 6–2–1938.
	7	2	2	....	Same as above.
	8	2	2	....	" " " .
	9	2	3[b]	....	" " " .
	10	2	2	....	" " " .
4	11	5	5	8.0 × 6.0 × 1.5	
	12	5	5	....	
5	13	5	5	2.5 × 1.8 × 1.1	
	14	5	2	1.3 × 1.8 × 0.4	Small, crowded by callus.
	15	5	4	1.5 × 0.9 × 0.4	" " " " .
	16	5	5	4.4 × 3.4 × 1.2	
	17	4	4	4.0 × 2.6 × 0.8	Began developing 7–3–1936.
6	18	5	5	5.5 × 2.9 × 1.7	
	19	5	5	5.0 × 3.1 × 2.2	
	20	5	5	5.0 × 2.4 × 1.9	

[a] Measurements of size are in the following order: longest horizontal axis × longest vertical axis × greatest distance from bark to outer margin of sporophore.

[b] The third tube layer was just beginning to form.

The upper surface of the sporophores had concentric ridges (Fig. 1, A) that corresponded approximately to the layers in the hymenophore, but the ridges were not always sufficiently distinct to be counted accurately. The size of the sporophores also was roughly correlated with the number of tube layers, but varied considerably between sporophores with the same number of layers.

The position of the sporophores on these 6 trees was restricted to branch scars as long as the trees remained alive (Fig. 1). One of the trees (No. 4), however, died in the spring of 1938 and was left standing. By September of the same year, many new sporophores had formed, irrespective of branch scars, on the trunk of the dead tree where the bark still remained rather firmly in contact with the sapwood. Another tree (No. 2) died and was cut down in the spring of 1937 and left lying on the ground, but no new sporophores formed on this dead trunk.

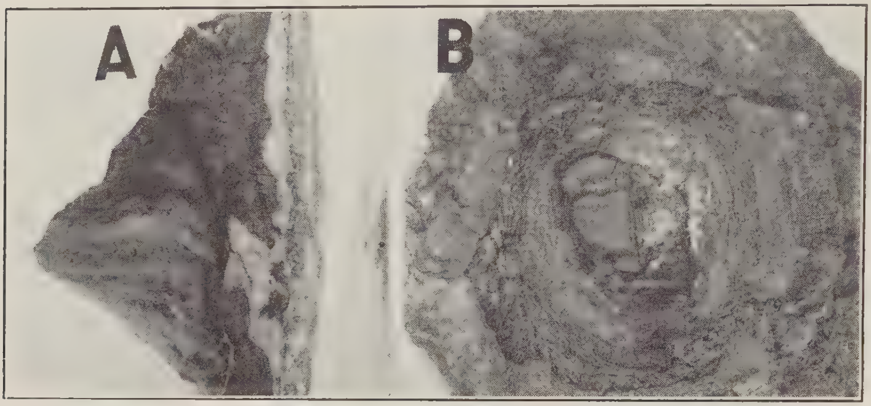

FIG. 1. A. Typical sporophore of *Fomes igniarius* formed at a branch scar on living aspen. This sporophore is 5 years old and contains 5 tube layers. B. Sporophore No. 15, which failed to produce a tube layer annually, possibly because it was almost enclosed by callus. The sporophore has been cut in half for the purpose of counting the tube layers. Both × 1.

The results of this study indicate that sporophores of *Fomes igniarius* normally form one tube layer in the hymenophore each year, except when their growth is restricted by surrounding callus or by other adverse factors.

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PHYTOPATHOLOGICAL NOTES

Ring Spot of Apricot.[1]—A destructive disease of apricot (*Prunus armeniaca* L.), characterized by irregular ring spots and blotches in the leaves and fruits, was observed near Palisade, Colorado, in 1935. At that time the condition was noted in a few trees of the Montgamet variety in several orchards. In the following years (1936–1939) the malady was observed to increase in the Montgamet, as well as in the Moorpark, variety.

Leaves exhibited irregular ring spots and a marked clearing of the principal veins. Such manifestations were most apparent on leaves of new shoot growth (Fig. 1, B). As the season progressed, the discolored tissues frequently became necrotic and crumbled away, giving the leaves a frayed and ragged appearance. Developing fruits appeared normal until approximately 2 weeks prior to ripening, about the middle of June. After this time the fruits began to exhibit protuberances, which often gave individual fruits a bumpy appearance. These excrescences were frequently depressed at the apex, giving a cratered effect (Fig. 1, A). The periphery of the papule was frequently discolored, forming a water-soaked green circular margin. As the fruits ripened, this water-soaked green color changed in most cases to a buffy citrine[2] and finally to a red-purple or red-brown hue. During the ripening process the affected fruits filled out, tending to eliminate their bumpy appearance but leaving reddish-brown blotches or ring spots (Fig. 1, C). Ripe fruits with such symptoms often showed cracks in the discolored areas. Sections cut through discolored portions of both green and ripened fruits revealed a necrotic condition of the tissues (Fig. 1, D). Such necroses followed the external form of the ring or blotch. The discolorations usually extended from $\frac{1}{16}$ to $\frac{1}{8}$ in. into the flesh, although necrosis occasionally was found to a depth of $\frac{1}{4}$ in. It has been observed that on any thoroughly diseased limb all the fruits showed such symptoms as those just described. Trees showing an infected limb or twig in one growing season invariably became completely diseased within one or two years. Infected trees have been observed for a period of 5 years, and in no instance have any of these trees showed recovery from the diseased condition. It has been noted that in orchards where diseased trees were eradicated in 1937 and 1938 there was no further spread of ring spot.

However, orchards containing diseased trees that were not removed showed an evident annual increase in the number of diseased trees.

Since repeated tissue plantings from necrotic tissues on nutrient culture media were negative, and the leaf symptoms were suggestive of virus infection, this latter possibility was investigated.

A study was undertaken to ascertain whether the disease could have been caused by the virus of peach mosaic. In the spring of 1936, cions taken from healthy Elberta peach trees were inserted into 5 large, bearing Montgamet apricot trees showing symptoms of the malady. Two peach cions were inserted into each tree. The cions made growth unions but showed no symptoms during the growing seasons of 1936 and 1937. In addition, in the fall of 1936, buds from diseased apricot trees were inserted into 20 Elberta peach nursery trees. In the spring of 1937 shoots from the inserted apricot buds showed leaf symptoms of the malady but no symptoms were observed on the peach growth. In like fashion symptoms were shown by the apricot growth during the seasons of 1938, 1939, and 1940, while none were exhibited by the peach growth.

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[1] Paper No. 121 of the Scientific Journal Series of the Colorado Agricultural Experiment Station.

[2] Ridgway, R. Color standards and color nomenclature. 43 pp., 53 colored plates. (Washington.) 1912.

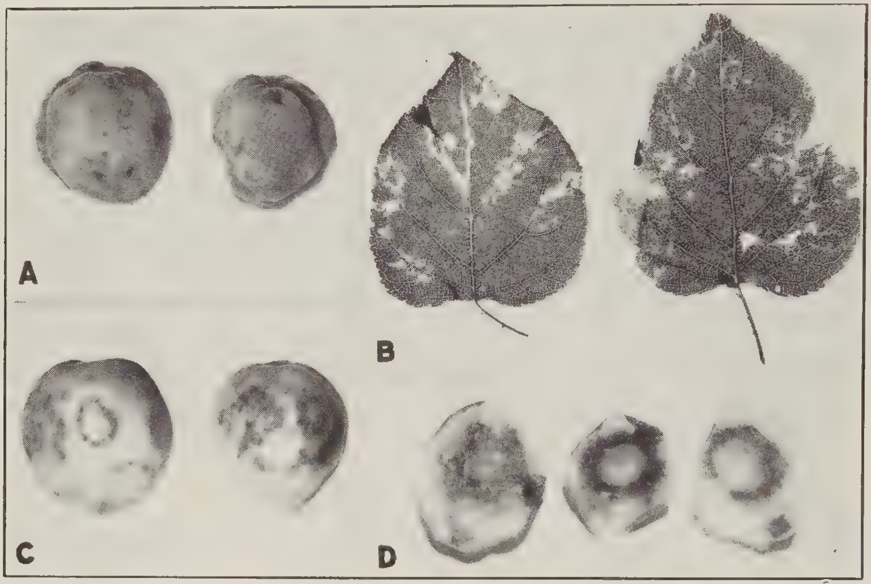

FIG. 1. Ring spot of apricot as expressed on the Montgamet variety. A. Diseased green fruit showing protuberances. B. Leaves from a diseased tree showing vein clearing and ring spots. C. Affected ripe fruit showing ring spots and blotches. D. Sections through diseased fruits showing necrotic areas in the tissue.

Since it was evident by the fall of 1938 that the apricot disease was not caused by the peach-mosaic virus, its possible infectiousness to apricots was investigated. Buds from Montgamet apricot trees showing symptoms of the disease were inserted into 6 healthy Moorpark apricot nursery trees. Two buds were inserted into each tree. The inserted buds made growth unions and showed characteristic symptoms in the spring of 1939 but no symptoms were apparent in the growth of the stock. However, in the spring of 1940 all the stocks produced leaves that showed typical ring spots and vein clearing.

To investigate possible fruit symptoms of the malady, buds from diseased Montgamet apricot trees were inserted into 5 bearing trees 20 to 25 years of age of the same variety in the fall of 1938. Five buds were inserted into each tree. The majority of the inserted buds made growth unions. During the growing season of 1939 no leaf or fruit symptoms were noted in the growth of the stocks. During the growing season of 1940, however, both leaves and fruit of all 5 trees showed the symptoms of the disease. Ten Montgamet nursery trees inoculated in similar fashion in the fall of 1939 failed to show symptoms in 1940. Both fruit and leaf symptoms, however, were evident on all 10 trees in 1941.

The foregoing studies indicate that ring spot of apricot is of a virus nature in which symptom manifestation is delayed for 2 years. Although there were some similarities in symptom expression, the malady appeared to differ somewhat from the apricot mosaic disease described by Atanasoff[3] as occurring in Bulgaria.—E. W. BODINE and W. A. KREUTZER,[4] Colorado Agricultural Experiment Station, Fort Collins, Colorado.

Wilt Resistance in F_1 Hybrid Watermelons.[1]—That wilt resistance existed in certain citrons (*Citrullus vulgaris* Schrad.) was first shown by Orton.[2] The edible varieties of *Citrullus* in general were wilt-susceptible. Porter and Melhus[3] found that crosses between wilt-resistant types of citron and wilt-susceptible edible melons yielded wilt-susceptible F_1 plants. As a result they emphasized the necessity of growing the F_1 progenies in soil that was not infested with *Fusarium bulbigenum* Cke. and Mass. var. *niveum* (EFS.) Wr. These findings and the fact that segregation of resistance and susceptibility occurred in the F_2 generation led to the assumption that wilt resistance was inherited as a recessive character and that edible melon varieties were homozygous for wilt susceptibility.

In 1937 an inbred selection (inbred 3 generations) from the variety Dixie Queen (wilt-susceptible) was crossed with a wilt-resistant inbred selection from a 3-way cross (Iowa Belle × Jugoslavia 7, backcrossed on Iowa Belle). The purpose of the cross was to develop a round, striped, wilt-resistant watermelon. The F_1 plants of the above cross were 70 to 85 per cent wilt-resistant.

In 1940, 420 hills of the F_1 seed resulting from the above named cross were planted in soil heavily infested with the wilt organism. Three to 6 seeds were planted per hill, but each hill was thinned to a single plant, when the plants were beginning to vine, in order that yield data might be collected. The plants that died subsequent to thinning were killed by the wilt pathogen. By September 1, 1940, 120 plants had been killed and 300 plants, or 71.4 per cent, were alive and bearing full-grown maturing melons.

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[3] Atanasoff, D. Mosaic disease of drupaceous fruit trees. Yearbook of the University of Sofia, Faculty of Agriculture 13: 9–42. 1934.

[4] The writers wish to express their appreciation to Dr. L. W. Durrell, Head of the Department of Botany and Plant Pathology of Colorado State College, for helpful advice and criticism.

[1] Journal paper No. J–925 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 71.

[2] Orton, W. A. On the breeding of disease resistant varieties. Inter. Conf. on Plant Breeding and Hybridization, Proc. 1902 (In Hort. Soc. N. Y. Mem. 1: 41–54). 1902.

[3] Porter, D. R., and I. E. Melhus. The pathogenicity of *Fusarium niveum* (EFS.) and the development of wilt resistant strains of *Citrullus vulgaris* (Schrad.). Iowa Agr. Exp. Stat. Res. Bull. 149. 1932.

In another experiment, F_1 seed from 12 additional crosses between wilt-resistant and wilt-susceptible lines was planted in a field heavily infested with the wilt organism. Only 20 hills from each cross were planted, but 2 of the 12 crosses were 80 per cent wilt-resistant. The parentage of these 2 crosses was as follows: Japan 7 × Thurmond Grey; Japan 7 × Dixie Queen. Wilt resistance in the other 10 crosses ranged from 50 per cent to zero, and the 35 hills of the wilt-susceptible Dixie Queen (check) were so susceptible that they all died. The experiment indicated that at least 2 of the hybrids were as wilt-resistant as the F_1 progeny in the previously mentioned experiment.

The fact that some F_1 progenies were wilt-resistant was not in accord with the findings of Orton,[4] who stated that "The first generation should not be exposed to the disease, as resistance may be recessive," and to the work of Porter and Melhus,[5] who found resistance to be a recessive character. In much of the earlier work citrons were used as the primary source of wilt resistance. It is conceivable that wilt resistance was inherited differently in crosses between the citron and watermelon than in crosses involving only watermelon varieties. Regardless of the genetical explanation for the inheritance of wilt resistance it appears clear that in crosses between wilt-susceptible and wilt-resistant edible lines of *Citrullus vulgaris*, the F_1 progenies may or may not be susceptible to *Fusarium bulbigenium* var. *niveum*.—AARON WELCH and I. E. MELHUS, Iowa State College, Ames, Iowa.

The Effect of Chemicals on Fusarium oxysporum cubense Growing in the Soil.[1]—Soil sterilization is practiced in greenhouses and in nursery beds for the control of soil-borne organisms. Brandes[2] used steam in the control of Panama disease and found that it killed out the fungus, but the expense was prohibitive. In order to reduce the spread of Panama disease some of the larger properties in Jamaica used a 1 per cent solution of formaldehyde to treat the banana suckers before they were planted in the field. Sodium nitrate in certain concentrations has been found to reduce the spread of Panama disease.[3] This study has been carried out to determine the per cent of chemical that is necessary to kill *Fusarium oxysporum cubense* in the soil.

The soil cultures used in the experiment were made up with a 0.5 × 8.0 cm. strip of banana leaf, 10 g. of air-dried clay soil, and 3 cc. of water. After the tubes were autoclaved the organism was planted and permitted to grow for 7 days. The chemical was added to soil cultures and allowed to remain for 24 hours before transfers were made to autoclaved banana-leaf tubes for growth observations. Growth could be observed in 24 hours in the checks, but the final reading was made on the third day after transfer.

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[4] Orton, W. A. On methods of breeding for disease-resistance. Proc. Soc. Hort. Science, 1907, p. 28.

[5] See footnote 3.

[1] Acknowledgment is due the Jamaica Banana Producers' Association for support in this research.

[2] Brandes, E. W. Banana wilt. Phytopath. 9: 339–389. 1919.

[3] Meredith, C. H. The effect of sodium nitrate on *Fusarium oxysporum cubense*. Phytopath. 31: 564. 1941.

The percentages given in tables 1 and 2 refer to percentages by weight of chemical to soil. Those of 40 or less in table 1 may be read as percentage of soil and chemical, or percentage of water and chemical. The 50 per cent results were obtained by mixing 1 part of soil culture and 1 part of chemical with 2 parts of water.

TABLE 1.—*The toxic and non-toxic points observed in chemical and soil-culture mixtures*

Chemical	Highest non-toxic *Per cent*	Lowest toxic *Per cent*
98. Ammonium carbonate	6.00	8.00
82. Bismuth nitrate	1.00	5.00
42. Bordeaux mixture	20.00	50.00
4. Boric acid	3.00	4.00
78. Cadmium chloride	20.00	50.00
34. Calcium hydroxide	8.00	9.00
33. Calcium oxide	6.00	8.00
24. Carbolic acid	0.37	0.75
23. Copper spray No. 1	8.00	9.00
39. Copper spray No. 3	30.00	50.00
40. Copper spray No. 4	20.00	50.00
5. Copper sulphate	5.00	6.00
73. Cupric nitrate	7.00	10.00
43. Ethyl mercury iodide	0.0005	0.0025
44. Formaldehyde	0.03	0.04
18. Ferric sulphate	7.00	8.00
49. Ferric nitrate	0.10	1.00
19. Hydrated lime	5.00	6.00
80. Iodine	Saturated solution	50.00
46. Lead acetate	30.00	40.00
35. Lime (air slaked)	10.00	20.00
2. Mercuric chloride	0.04	0.05
45. Mercuric oxide	0.10	1.00
37. Mercurous chloride	0.50	1.00
44. Mercury protonitrate	0.01	0.05
15. Methylated spirits	14.00	15.00
62. Oxalic acid	4.00	6.00
29. Paris green	20.00	50.00
68. Potassium bisulphate	10.00	20.00
70. Potassium chromate	20.00	30.00
69. Potassium cyanide	0.10	1.00
36. Potassium dichromate	0.50	1.00
94. Potassium hydroxide	4.00	6.00
92. Potassium iodide	9.00	10.00
6. Potassium permanganate	3.00	4.00
71. Potassium sulphacyanide	8.00	9.00
76. Potassium sulphocyanate	20.00	50.00
3. Silver nitrate	0.02	0.03
26. Sodium carbonate	20.00	30.00
95. Sodium hydroxide	0.10	1.00
41. Sodium nitrite	0.10	0.50
11. Sodium silicate	12.00	20.00
61. Stannous chloride	4.00	5.00

There was considerable variation in the ability of the chemicals to retard the growth of the transferred spores before the death point was reached.

TABLE 2.—*Chemicals that are not toxic to Fusarium oxysporum cubense in a 50 per cent soil-culture mixture*

Alum
Aluminum silicate
Aluminium sulphate
Ammonium nitrate
Ammonium oxalate
Ammonium sulphate
Antimony
Antimony sulphide
Antimony tersulphide
Barium chloride
Calcium chloride
Calcium sulphite
Chromium sulphate
Cobaltous chloride
Copper spray No. 2
Iron oxalate
Iron sulphide
Indigo
Lead arsenate
Lead chloride
Lead nitrate
Lead oxalate
Lead sulphate
Lead sulphide
Litharge
Magnesium ammonium phosphate
Magnesium carbonate
Magnesium oxide
Magnesium silicate
Magnesium sulphate
Manganese dioxide
Manganese sulphate
Nickel sulphate
Potassium antimoniate
Potassium bicarbonate
Potassium bromide
Potassium chlorate
Potassium chloride
Potassium ferricyanide
Potassium ferrocyanide
Potassium nitrate
Potassium sulphate
Protosulphate
Red lead
Red iron oxide
Sodium acetate
Sodium chloride
Sodium nitrate
Sodium phosphate
Strontium chloride
Strontium nitrate
Sugar
Sulphur
Superphosphate
Zinc carbonate
Zinc oxide

A 30 per cent mixture of sodium bicarbonate did not kill the fungus. Of the 100 chemicals, 43 killed *Fusarium oxysporum cubense* in the strength mixture given in table 1. In 56 cases the fungus was not killed by a 50 per cent mixture.—CLIFFORD H. MEREDITH, Glenleigh Laboratory, Friends College, Highgate, Jamaica, B.W.I.

Growth of Diplodia Macrospora in Media Containing Pure Biotin.—In 1940 Margolin[1] reported that the growth of *Diplodia macrospora* Earle required "biotin or a biotin-like substance." In 1941[2] it was discovered that the substance necessary to a culture medium containing mineral salts and dextrose is produced by the closely related *D. zeae* (Schw.) Lév. As is well known both fungi occur commonly in causal relationship to corn ear rots, although *D. macrospora* is of more limited distribution, being confined largely to warmer regions.

During the past winter it has been found that the addition of pure crystalline biotin-methyl ester[3] to the basal medium supported growth of *Diplodia macrospora*. Some growth was observed with 0.5 γ of the biotin-methyl ester per liter. Much better growth occurred with the addition of 2.0 γ per liter.—NEIL E. STEVENS and R. A. CHAPMAN, University of Illinois.

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[1] Margolin, A. S., Proc. W. Va. Acad. Sci., 14 (Keyser), in W. Va. Univ. Bull., Ser. 41, No. 4–11, 1940.

[2] Stevens, Neil E. and W. E. Wilson. Science 93: 458–459, 1941.

[3] Product of the S.M.A. Corp., Chagrin Falls, Ohio.

BOOK REVIEW

OSCAR KRISEN BUROS. *The Second Yearbook of Research and Statistical Methodology.* The Gryphon Press. Highland Park, New Jersey, 1941. pp. XX + 383. $5.00.

The yearbooks of Research and Statistical Methodology, prepared by Prof. Oscar Krisen Buros, are unusual, if not unique, consisting as they do of excerpts of reviews of numerous books appearing in the principal scientific journals. The first yearbook was published under the title "Research and Statistical Methodology Books and Reviews of 1933–38." The second, in part, duplicates the first, "since it is the yearbook policy to continue listing books in successive yearbooks as long as new reviews appear." A total of 1,652 reviews of 359 books or an average of slightly more than 4.5 per book are listed, and excerpts from most of them are included. These reviews were originally published in 283 journals.

Most of the reviewed books deal with statistical methods, in general, or as related to particular fields of inquiry. Economics, education, and psychology are most extensively represented, probably because statistical methods are widely used in these fields and many books explaining their use have been written. Agriculture and biology are represented by a considerable number of books, though relatively few as compared with the above; and chemistry and physics scarcely at all. Several reviews pertain to books dealing with science in general and a few to such miscellaneous subjects as field plot technique, report writing, and general research methods in special fields. The type and printing are excellent. There is included a list of cooperating journals, a periodical directory and index, a publishers' directory and index, an index of names, and a classified index to books.

The need for this book, as seen by the author, arises from the "tremendous advances in statistical theory" that have been made in recent years, the multiplicity of books dealing with statistical methods, and the fact that many of the authors, as well as teachers and investigators, who use these books are "ignorant of recent developments." Many readers will agree with this analysis of the present situation, though probably a good many will hesitate to depend fully on book reviews to indicate with books are least likely to be out-of-date or misleading. Granting that this is an adequate method, it must be admitted that a good job of collecting and excerpting reviews, and arranging them, has been done. Also, no one after reading them will deny that they provide a good starting point for more careful consideration. For the librarian, teacher, or investigator who wishes to add to his collection of books on statistical methods, the present volume should be of much use.

The volume may be useful in other ways, especially for those, if there be such, who have become infatuated with statistical methods. A consideration of the reviews will show beyond doubt that, even though the use of statistical methods will "lead to certainty," as one author has recently stated, it makes a great deal of difference which particular statistical authority one leans upon for advice. Prof. Buros' belief that discrimination is highly desirable appears to be well supported by a recent article by Hotelling to which the interested reader is referred (The Teaching of Statistics, by H. Hotelling. The Annals of Mathematical Statistics. Vol. XI, No. 4, Dec. 1940, pp. 445–470).—S. C. SALMON, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

MORTALITY OF THE RED SCALE ON CITRUS THROUGH INFECTION WITH A SPORE–FORMING BACTERIUM

V. P. SOKOLOFF[1] AND L. J. KLOTZ[2]

(Accepted for publication June 26, 1941)

Bacterial parasites of the California citrus red scale, *Aonidiella aurantii* (Mask.), have, so far as we know, never before been isolated or studied. It appeared of interest, therefore, to devote some attention to a microorganism recently proved to be a pathogen of red scale on citrus and an occasional inhabitant of dead citrus scale in some orchards. This organism was first isolated from soil in connection with denitrification studies and grown on chitin, cellulose, and a large number of organic media. It is tentatively designated Bacillus "C."

Under certain laboratory conditions it has been found possible to bring about a wholesale invasion and death of the red scale by Bacillus "C," especially on lemons.

THE MICROORGANISM

Bacillus "C" is a large (6 by 1¼ μ) Gram-positive motile rod, which forms spores in the equatorial position. Its motility is contingent upon the presence of clumps of several polar flagella and is lost, as a rule, after a few days of growth on the ordinary laboratory media. The microorganism grows singly, in couples joined end to end, and in chains of 4 or more (Fig. 1).

Bacillus "C" is an aerobe. It can grow throughout a number of liquid media in the presence of either nitrate or nitrite, provided oxygen is not entirely excluded, as in cotton-stoppered flasks or test tubes. On Spray anaerobic plates, Bacillus "C" develops no growth, even in the presence of nitrate. Ammonia and a still unidentified nitrogenous substance frequently appear to be the products of the reduction of nitrate or nitrite. Within a rather narrow optimum, on the other hand, nitrates appear to be ultimately reduced to the gaseous forms of nitrogen.

On aerobic plates, Bacillus "C" grows within the entire pH range studied, that is, from pH 4 to pH 9.5. In liquid cultures an optimum in the vicinity of pH 7.5 is suggested, while growth is limited to the active acidity range of pH 6 to pH 9.3. The optimum temperature for the growth of Bacillus "C" in the suspensions of chitin is in the vicinity of 30° C. It is able to grow, however, at 12° and at 40° C.

Bacillus "C" was originally isolated from the following enrichment medium:

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[1] Junior Chemist, University of California Citrus Experiment Station, Riverside, California.

[2] Associate Professor of Plant Pathology, University of California Citrus Experiment Station, Riverside, California.

Filter paper, Whatman No. 42, dispersed in the Waring blender	10 per cent
Potassium nitrate, Baker's	1 ‘‘ ‘‘
Potassium hydrogen phosphate, Baker's	1 ‘‘ ‘‘
Magnesium sulphate heptahydrate, Baker's	½ ‘‘ ‘‘
Calcium carbonate, Mallinkrodt's	2 ‘‘ ‘‘
Redistilled water (all-glass still)	

Inoculum: 5 grams of surface soil from the Citrus Experiment Station.

The original enrichment culture was incubated in a 50-cc. glass-stoppered Pyrex bottle at 28° C. for about 1 week. Transfers were made into wide-mouthed 500-cc. Pyrex Erlenmeyer flasks stoppered with cotton, containing 300 cc. of sterile medium of the same composition as the enrichment culture (Fig. 1, A). After 2 successive transfers, the mixed culture was plated on 2 per cent agar, containing the same filter paper and salts as the original enrichment medium. At the same time platings of the mixed culture were made on peptone-yeast agar. Among the organisms developed on these plates were two bacteria that grew equally well on filter paper, peptone-yeast, and chitin agar. One of these, a spore-former, was chosen for further observation.

In general outline and in manner of sporulation there appear to be certain resemblances between Bacillus ‘‘C’’ and the microorganism described by Dutky (3).

EXPERIMENTAL PROCEDURE

Field lemons, infested with the red scale, were used almost exclusively in the investigations here reported. These lemons were collected in 2 different orchards: one in the vicinity of Corona, California; the other in the City of Riverside. Lemons from the latter grove had not been fumigated nor otherwise treated for the red scale during the preceding 10-year period. Hand-picked lemons were brought into the laboratory and carefully washed with soap. It was found necessary also to wash them for 1 or 2 minutes in 0.1 per cent copper sulphate solution to minimize the importance of fungi as an interfering factor. Neither soap nor copper sulphate, however, was found to remove or destroy all of the indigenous bacteria;[3] hence it cannot be stated that the work presented here was done under sterile conditions.

Bacillus ‘‘C’’ was conditioned for the infection in the following manner: 48 hours before the experiment, 500-cc. aliquots of peptone water,[4] in a 1-liter Pyrex Erlenmeyer flask stoppered with cotton, were inoculated with a loopful of bacteria from a pure-culture slant. When suspensions of the

[3] One of these indigenous bacteria in particular appeared to be interesting on account of its ability to grow rapidly on chitin, and to produce a characteristic dark-yellow pigment under a variety of conditions. It was possible several times to isolate this pigment producer from crushed bodies of the insects that were already dead and dried on the fruit. It did not prove, however, to be pathogenic to the normal scale under the conditions of the present study.

[4] Composition of the peptone water:

Proteose peptone, ‘‘Difco’’	1.0 per cent
Potassium nitrate, Baker's	0.1 ‘‘ ‘‘
Potassium hydrogen phosphate, Mallinkrodt's	0.1 ‘‘ ‘‘
Magnesium sulphate heptahydrate, Baker's	0.05 ‘‘ ‘‘
Tap water, pH 7.5–7.6 (glass electrode)	

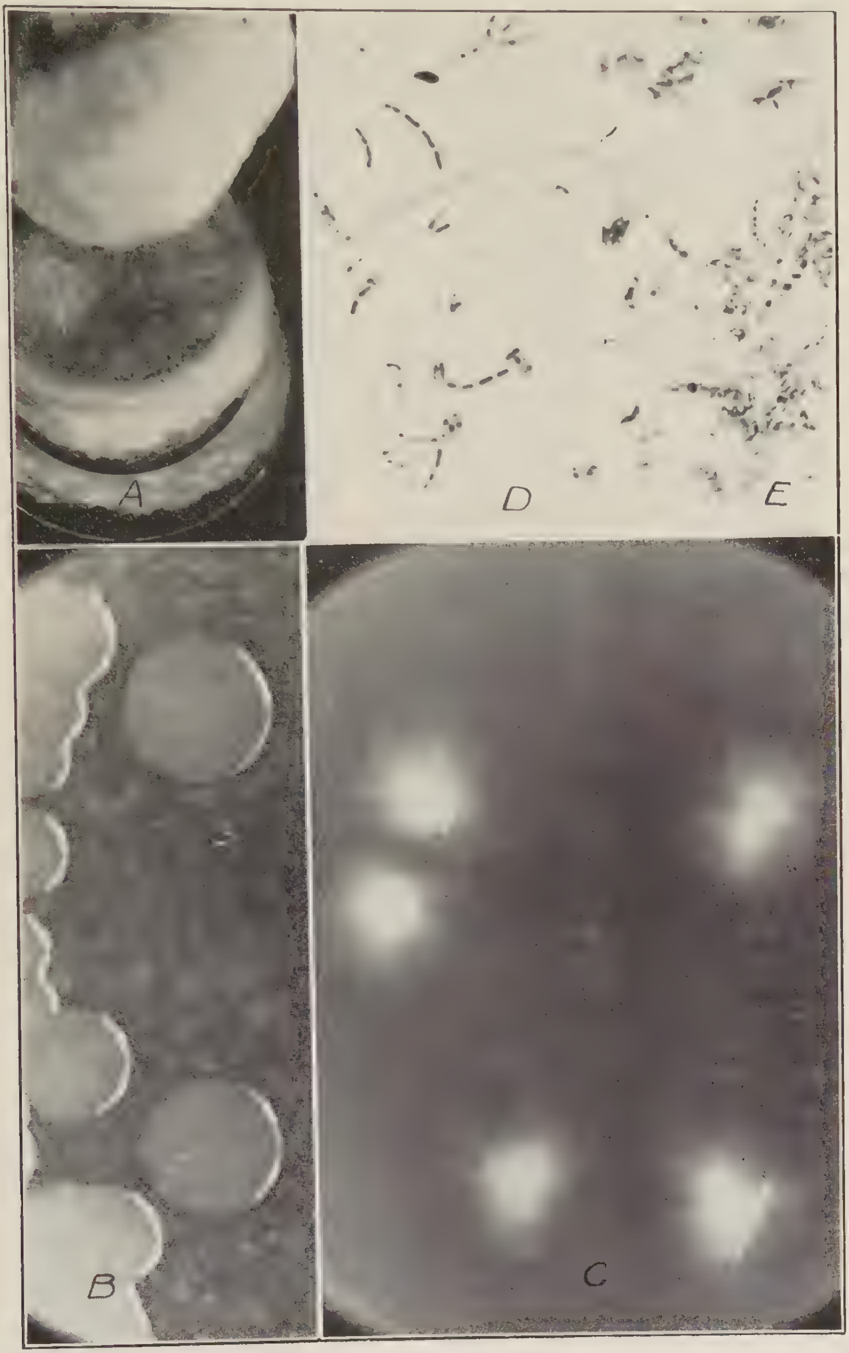

FIG. 1. Bacillus "C." A. Active transfer from the filter paper nitrate-enrichment medium. B. Two-day colonies on peptone agar. ×12. C. Two-day colonies on citrate-asparagin agar. ×12. D. From peptone-nitrate broth (Gram stain). ×1,500. E. Unstained spores from chitin agar. ×1,500.

bacillus in tap water or in buffered distilled water were used, 48-hour-old aerobic growth on peptone-agar plates was scraped off and suspended, under sterile conditions, in 500-cc. aliquots of water.

It was found desirable to maintain both the fruit and the bacterial cultures at the same temperatures (28° C.) for approximately 6 hours before the experiment. Single fruits were then placed for variable periods of time in beakers containing either peptone-water cultures or suspensions of the bacillus in sterile tap water.

Parallel ‘‘treated controls,’’ in which sterile peptone broth or sterile tap water only was used, were provided for all tests in all cases. ‘‘Untreated controls’’ were likewise maintained. These were fruits incubated alongside infected and ‘‘treated control’’ fruits, without any treatment whatsoever.

It was found that in order to obtain a satisfactory mass infection of the scale, 2 to 4 hours' immersion was necessary. In the majority of cases, however, a 16-hour immersion was preferred because of the consistency and the reliability of the results ensured by the longer wetting period. At the expiration of the period of immersion, the controls, as well as the infected fruits, were removed from the beakers by means of crucible tongs and placed in the incubator at 28° C. for variable periods of time. Under these conditions, it made no difference whether in order to obtain satisfactory results the fruit was kept in a moist chamber or in the open air. When the fruits were immersed for shorter periods, however, the moist chamber appeared to be indispensable. At the conclusion of the incubation period, all adult female insects were removed from the fruits and examined under the microscope, individually, for general appearance, movement of the pygidia, and a few tentatively defined symptoms of the infection (Table 1),

TABLE 1.—*Visible signs of infection of citrus with Bacillus ‘‘C’’*

Insects	Total number examined	Adult insects			Crawlers	
		Per cent showing				
		Browning	Distortion of pygidia	Disintegration	Total number examined	Per cent mortality
Infected with Bacillus ‘‘C’’	1120	66	56	32	965	98
Controls (treated)[a]	575	47	56	22	191	57
Controls (untreated)	602	18	28	11	353	25

[a] Treated in the same manner as the infected ones except for the absence of Bacillus ‘‘C.’’

When chemical analyses of normal and of infected adult insects were undertaken (in lots of 100), every insect in the lot was examined under the microscope prior to the analysis in order to ensure, so far as possible, complete uniformity in the condition of the insects. Aqueous extracts of the insects chosen for the analysis were made in the following manner: One hundred adults were crushed with about 5 cc. of distilled water and 1/10 g. of copper sulphate. About 50 cc. of distilled water was then added, and the extract was boiled in an Erlenmeyer flask under a reflux condenser for 2 min. Approximately 1/10 g. of the secondary potassium phosphate was added after the digestion; also 1/10 g. of calcium carbonate and a trace of magnesium oxide. One g. of "Norit" activated carbon was finally introduced, and the mixture was brought rapidly to the boiling point. The hot extract was filtered through Whatman No. 42 filter paper. The residue on the filter was washed with about 100 cc. of boiling distilled water. The combined filtrate and washings were cooled and made up to 250 cc. with distilled water. Aliquots of this colorless transparent filtrate were taken for the colorimetric determinations of nitrate and nitrite. Nitrite was determined by the Griess reagent, following the usage of Elema (5). Nitrate was determined by the official phenoldisulphonic-acid method (1).

Total nitrogen was determined on separate lots of the insects by means of the Gunning-Hibbard modification of the Kjeldahl procedure (1). The acid digestion was made in micro-Kjeldahl flasks. For the distillation, the ordinary Kjeldahl apparatus was used.

THE INFECTION

Examinations of the infected adult red scales often show Bacillus "C" adhering to the waxy membrane of the lower margin of the insect, especially in the proximity of the pygidial cavity. When insects, detached from the fruit, are wetted with suspensions of the bacillus, it is sometimes possible to observe the latter penetrating the pygidial cavity and apparently entering the insect through the oviduct.

Crawlers are attacked by the bacillus with far greater rapidity than the adult insects. Clumps of the bacilli are frequently formed in the vicinity of the joints of the crawler's legs, and in less than 15 minutes after the formation of a well-defined clump, the crawler succumbs. In the case of the adults, on the other hand, pygidial movements cease only after approximately 25 minutes of the contact with the bacillus.

After but a few days of incubation in the dry at 28° C., a large proportion of the infected adult insects exhibit a rather characteristic browning of the ventral surface. This browning is sometimes limited to the areas adjoining the pygidial cavity or to the vicinity of the mouth. Brown spots may be observed occasionally on the prosomatic lobes and on the surface of unhatched embryos or eggs inside the insect.[5] An expulsion of dead crawlers and embryos, especially on grapefruit, takes place occasionally during this stage of the infection. There is a general darkening in the color of the infected insects that is visible to the naked eye (Fig. 3). Pygidia, viewed under the microscope, sometimes appear distorted, that is, twisted, collapsed, irregularly swollen, or otherwise deformed.

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[5] Similar browning of the ventral surface of the insect also was found to be a consequence of 1 hour's immersion in 0.1 M solution of hydroxyl amine containing pH 7.5 phosphate buffer. Comparable treatments with nitrites, nitrates, and ammonium salts caused no browning. Neither was it observed in the insects treated with hydrogen sulphide or hydrogen cyanide. Natural illuminating gas, however, and 5 days' storage over pyrogallol in sealed desiccators were found to produce browning that resembled, superficially, the browning caused by the bacillus.

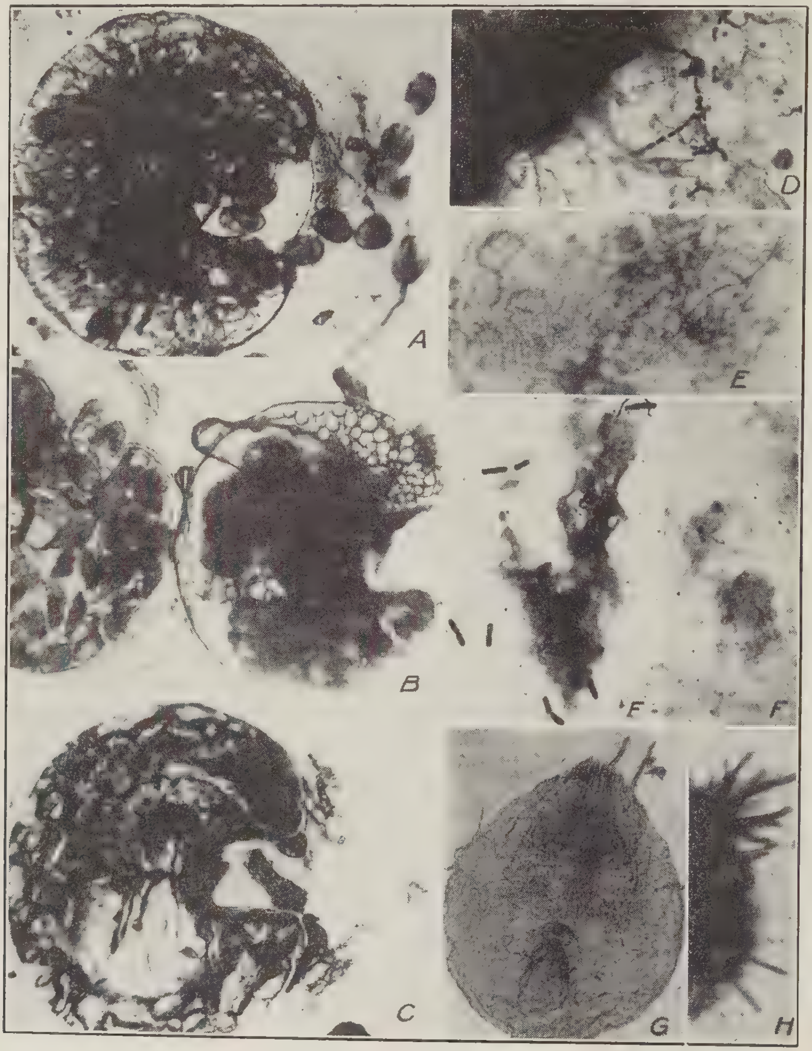

FIG. 2. A. Normal adult female citrus red scale with crawlers (ventral view). ×64. B. Infected adult female with dead crawlers (ventral view). Formation of gas within the insect. ×64. C. Final disintegration of the insect. ×64. D. Bacillus "C" invading the outer margin of the insect (Gram stain). ×1,000. E. Bacillus "C" inside the infected insect (smear preparation; Gram stain). ×250. F. Bacillus "C" in the crushed contents of the insect (Gram stain). ×1,500. G. Fungus (*Cladosporium* sp.) conidiophores growing from body of red scale (still alive). ×64. H. Conidiophores. ×140.

After 5 or 10 days of incubation, it is frequently possible to observe a general disarrangement of the internal structure of the insect. Gas bubbles

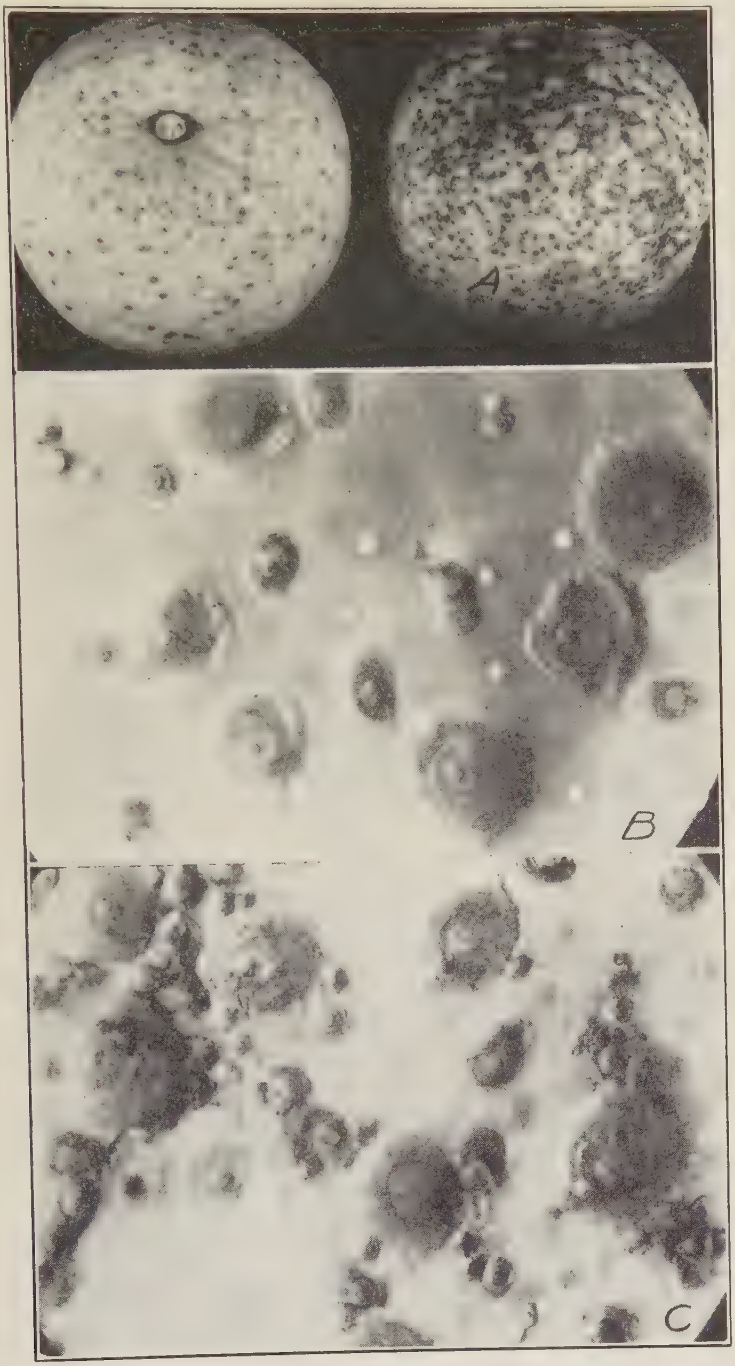

FIG. 3. Red scale on oranges: A. Effect of 16-hour immersion in a culture of bacillus "C" and of subsequent dry incubation at 28° C. for 2 weeks (right); parallel untreated control (left). B. Surface of the untreated control orange (note the white caps). C. Surface of the treated orange. ×14.

can sometimes be seen under the microscope as they are evolved from the infected body. At this stage the saprophytic fungi (facultatively parasitic) may invade the insect, unless special precautions were taken to minimize their numbers on the fruit (Fig. 2, G and H). Despite the disarrangement and apparent decomposition, the body of the insect appears relatively turgid. The drying of the dead insects takes place gradually and requires approximately 3 weeks.

There is a good deal of deviation from the course of the disease and death here tentatively charted. Some individuals, although obviously infected, exhibit but one or none of the symptoms. Others pass the decomposition stage before any browning is visible. In other words, a satisfactory definition of the symptoms of the infection or an establishment of a sequence of the successive stages of the disease leading to the death of the insect cannot yet be made with precision. There appears to be a considerable degree of variability in the nature and order of symptoms and stages, not only in the insects on different hosts but in the insects on one and the same fruit or leaf, often situated within a few millimeters of each other. It was found possible, nevertheless, by examining large numbers of adult insects systematically, to make certain comparisons between the healthy and the diseased, on the basis of their appearance under the microscope (Table 1). A summary of these comparisons includes insects from lemons, oranges, grapefruit, and a relatively small number from lemon leaves. In these comparisons, no attempt is made to differentiate between the symptoms observed on various hosts or to allow for the variations in the method of the infection (by dipping, immersion, or spraying), as it appears for the present that small differences due to these variables are overshadowed by individual variations between the insects on the same host.

After the infected red scale has been incubated for several days at 28° C., it is possible, in the smear preparations, to demonstrate abundant growth of the bacillus within the insect. Generally speaking, during the first few days of the infection single cells of the bacillus are preponderant in the invaded insect. At a later period an abundance of chains may be noted. Sporulation has been observed at all times, becoming prominent 1 or 2 weeks after the infection.

Thus far we have no evidence of phagocytosis. Nor is there yet any evidence of morphologically different stages of the bacillus, once it becomes established within its host. The microorganism appears morphologically stable or true to its form and manner of growth under a variety of the environmental conditions (Fig. 2).

IMMERSION EXPERIMENTS

While it is possible to bring about individual infection of red scale by the bacillus, regardless of the latter's age, by applying copious quantities of the bacterial suspension to individual insects by means of a loop or a pipette, successful mass infection appears to be contingent upon a number of considerations:

Motile cultures of the bacillus are preferable to nonmotile. On aerobic plates, as well as in liquid media, the bacillus becomes largely nonmotile after 1 or 2 days. Young cultures were used accordingly in all our experiments. In the instance of the aerobic plates, the most satisfactory motile cultures were obtained on 4 per cent Liebig's Extract of Beef (manufactured by Oxo Ltd., London, Eng.).

Satisfactory wetting of the fruit with the bacterial culture or suspension presents a problem of some difficulty. This mechanical difficulty may be overcome eventually by use of a suitable wetting agent. Such wetting agents as blood albumin or casein offer a degree of promise, but some that are most effective appear to be incompatible with the bacillus. In our preliminary studies, therefore, we preferred to obtain the desired result by prolonging the period of immersion rather than by the use of wetting agents.

Too rapid a drying of the treated fruit militates against successful mass infection of the insects. By extending the period of the immersion to several hours, however, this difficulty is rendered insignificant. In other experiments in which the contact of the fruit with the bacilli was relatively brief, an almost complete failure of the mass infection was observed when the fruit was allowed to dry within less than 2 hours' time.

Finally, periodic reisolations of Bacillus "C" from sterile soil[6] were found desirable to maintain the ability of the bacillus to destroy the scale. A somewhat arbitrary criterion served as a sign of the need for reisolation, namely, failure of the pure culture of the organism to grow throughout liquid media in the presence of potassium nitrite. Its ability to grow anaerobically in the presence of nitrite was commonly restored upon its reisolation. There was likewise a parallelism between its pathogenicity to the red scale and its ability to employ nitrite as a hydrogen acceptor.

Immersion for 1 or 2 hours at 28° C. was often sufficient in itself to infect and destroy every one of the adult females on lemons, regardless of the total number on a single fruit. Our preliminary studies were limited nevertheless to the 16-hr. immersion treatment as a method of ascertaining pathogenicity of the bacillus to the citrus red scale.

A summary of the results of these treatments is given in table 2. Death or survival of the insects was ascertained at the end of 2 to 4 weeks' incubation following the immersion. The exact time of the count was largely determined by the "controls." A large-scale emergence of crawlers and the subsequent appearance of "white caps" on the control fruits (there were no active crawlers or "white caps" on the treated fruits) was taken as indication of the timeliness of the comparative counting. In determining the survival of the insects, movement of the pygidia alone was not regarded as a sufficient basis. Any insect appearing free from the signs of the decomposition was deemed "living." On the other hand, any insect showing

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[6] Five g. of garden soil moistened with 5 cc. of water were sterilized at 16 lb. pressure in cotton-stoppered test tubes for several successive 1-hr. periods, 2 days apart, until proved sterile by incubation with peptone-yeast broth. Soil extracts prepared in a number of different ways could not be substituted successfully for the soil.

TABLE 2.—*Mortality of adult red scale on lemons after immersion for 16 hours in cultures or suspensions of Bacillus "C"*

Treatment	Total adults	Mortality		
		Low	High	Average
Bacillus "C" in tap water	432	98	100	99
Sterile tap water only	460	36	79	55
Bacillus "C" in peptone water	652	98	100	99
Sterile peptone water only	553	35	89	61
None	887	29	60	37

signs of drying or disintegration, whether or not accompanied by browning or distortion of the pygidia, was regarded as dead.

Our understanding of the pathogenicity of the bacillus to the red scale is complicated by the fact that the fruits were not and could not be made sterile at the time of the experiment. For this reason, the so-called treated controls are not controls in the true sense of the word. In many instances, when these controls were found to harbor bacteria apparently identical with Bacillus "C," the main difference between the treated and the control fruits, under conditions of the immersion, might have been merely in the size of the inoculum. On the other hand, it is conceivable that the lethal effect of the bacillus may be enhanced by some other biological factors indigenous to the fruit or the insect. Further work may serve properly to ascertain the existence and relative importance of such factors.

DISCUSSION

It is not possible to suggest at this time any definite mechanism underlying the pathogenic effect of the bacillus on the insect, or to surmise the nature of the toxic principles at work. The data presented here may, nevertheless, provide a basis for a speculative discussion of at least one of the many possibilities in this regard.

Aqueous extracts of normal adult insects contain, as a rule, measurable quantities of nitrates. The nitric nitrogen constitutes somewhat less than 10 per cent of the total nitrogen.[7] Extracts of infected insects contain an appreciably lower amount of nitrate. The order of magnitude of the change in the nitrate concentrations is indicated in table 3.

It is evident from the available data that the slight increase in the soluble nitrite, in consequence of the infection, is insufficient to account for the loss of nitrate. Colorimetric measurements of ammonia in comparable extracts offered no evidence of any regularly significant change in the extractable ammonia of the insects. While there is as yet no direct basis to suggest

[7] Total nitrogen in the normal adult red scale from lemons was found to be 1060 and 1050 micrograms N per 100 insects. Nitric nitrogen constituted 104 and 110 micrograms N per 100 insects. For strictly comparable lots of the insects infected with the bacillus, the figures were, in micrograms N per 100 individuals, 1020 and 1020 for total N, and 47 and 45 for nitric N. The live weight of 100 normal adults is approximately 23 milligrams; of 100 dead adults it is approximately 18 milligrams.

Haas (6) reports more than 6 per cent total N in the red-scale insects, dry basis. Our results imply a slightly higher figure.

the possibility of the well-known reaction between the nitrite and the alpha amino acids of the insect, this possibility should be at least considered. Nor can we disregard the potential toxicity of the substances that may be formed in the course of the further reduction of nitrates, once this reduction could be proved to take place in the infected insect.

Immersion of lemons supporting the scale in buffered distilled water suspensions of Bacillus "C" was often accompanied by the appearance of measurable quantities of nitrite in the suspensions. These suspensions were free from both nitrate and nitrite before the fruit was introduced, and there was neither nitrate nor nitrite in the controls where only buffered distilled water was used. In view of a considerable variability in the amounts of nitrite found in the experiments of this type (ranging from a trace to several parts per million), it is not feasible, for the present, to regard this observation as an aid in the interpretation of our other results.

TABLE 3.—*Determinations of nitrate and nitrite in aqueous extracts of normal and infected adult red scale on lemons*

Insects[a]	Number of analyses	Nitrate (micrograms N)			Nitrite (micrograms N)		
		Low	High	Average	Low	High	Average
Normal	13	90	135	105	0.0	3.0	0.6
Infected with Bacillus "C"	7	0	85	56	0.5	2.8	2.0
Dead and dry, 4 weeks after the infection	6	0	35	12	0.0	0.3	0.1

[a] Tested in lots of 100.

The nitrate content of insects subjected to hydrogen cyanide fumigation or exposed to natural illuminating gas in sealed desiccators does not differ from that of untreated insects. After 5 days of storage over pyrogallol in sealed desiccators, on the other hand, the extractable nitrate was in comparison with that of untreated insects (185 and 172 micrograms as against 100 micrograms N per 100 adult insects). Since the insects derive their nourishment from the albedo cells of their host, containing, among other things, nitrate,[s] its presence in their body fluids is scarcely surprising. The remarkable ability of the scale to survive for more than 24 hours in the absence of oxygen (4) may not be unrelated to the presence of nitrate in their substratum. Since they cannot survive indefinitely, however, under such conditions, we may venture a speculation that, while nitrate itself may not be toxic to the insects, the products of its reduction may be toxic, directly or indirectly. For example, hydroxyl amine is a known inhibitor of the cytochrome-A_3 (2). There is a similarity in the appearance of the insects that died in consequence of their infection with Bacillus "C" and of those that died after several days' storage over the pyrogallol, as well as of those whose death followed their brief immersion in hydroxyl amine.

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[s] The presence of nitrate in the extracts of both albedo and flavedo cells of lemons was demonstrated by the brucine and the phenoldisulphonic-acid methods, as well as by the m-xylenol method (7). Significant quantities of ammonia were formed, also, upon the addition of zinc and ferrous sulphate to ammonia-free extracts (previously distilled in the presence of alkali). Because of the well-known limitations of all these methods, when applied to plant material, only the order of magnitude of the concentrations of nitrate in the albedo cells can be indicated. It appears to be in the vicinity of 60 to 100 p.p.m. N. Grapefruit albedo cells contain much less nitrate.

SUMMARY

A denitrifying bacillus, unidentified with any of the known microorganisms, is able, under laboratory conditions, to invade and destroy the adult red scale on lemons. Infection and death of the scale is accompanied by a significant decrease in soluble-nitrate content. It is possible that the lethal effect of the bacillus is related to the reduction of nitrate inside the insect.

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PRESTORAGE DISINFECTION OF NARCISSUS BULBS

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The control of narcissus basal rot, caused by one or more specialized forms of *Fusarium bulbigenum* Cke. and Mass., in regions where environmental factors during the growing and bulb-storage seasons are conducive to this disease, requires periodic sanitizing treatments of the bulbs sufficient at least to prevent the superficial contamination and latent infections with this organism from becoming active infections (1, 3, 4, 5, 7, 8). For the purpose of bulb sanitation, chemical treatments might be given either at planting time, which follows the summer storage period, or between the time of harvesting the bulbs and their placement in storage (3, 4). Practical considerations, especially the greater susceptibility of the recently harvested bulbs to chemical injury, make the pre-planting application the preferable and, in commercial bulb culture, the only time for giving these treatments. Generally, a brief immersion in a suspension or a solution of one of the organic mercury disinfectants is employed in commercial narcissus culture in the United States (4, 8).

When a thermal bath is required to disinfest the bulbs of animal parasites, such as nematodes, larvae of narcissus flies, and mites, the mercury-dip treatment is supplanted by a combination warm-water and chemical treatment, for which formaldehyde often is used in place of mercury compounds because of its demonstrated effectiveness against the bulb nematode, *Ditylenchus dipsaci* (Kühn) Filipjev (1, 3). Marked improvement in health and productiveness of treated bulbs has generally resulted from these treatments, and commercial narcissus growers have acquired enough experience in the adaptation of treating dates and concentrations to the requirements of particular varieties so that chemical injury to the bulbs seldom occurs.

As basal rot is primarily a storage disease it would seem *a priori* that a sanitizing treatment applied at the beginning of the storage period should be more effective than one given prior to planting in the preceding autumn, which antedates the storage period by 8 to 10 months. Experiments have indeed shown that basal rot during storage can be almost entirely prevented by previous chemical treatment, but usually at the cost of more or less damage to growth and flowering in the subsequently planted bulbs. This injury occurs in two forms or degrees of severity, (a) that immediately visible in discolored and indurated bulb scales or necrotic pitting especially of the root plate, and (b) deformity of the flower and sometimes of the shoot during the ensuing growth of the bulb. The shoot malformation simulates the injury caused by ethyl mercury phosphate in wheat and corn seedlings (2, 6), and the floral deformity in narcissus is so characteristic that a definite diagnosis of mercury injury to the bulbs usually can be made from inspection of the flowers. Because of this flower injury, growers have seldom been willing to adopt prestorage treatments, even though the alternative risk of losing untreated bulbs from storage rot is also great. However, experimental efforts have been continued to adapt prestorage chemical treatments of narcissus bulbs to the conflicting requirements of (a) prompt application following harvesting in order to prevent superficial infections from becoming deep-seated, and (b) minimal toxicity to the bulbs, susceptibility to which declines rapidly after the bulbs become dry.

A variety of materials thought to have value for this purpose have been tested at different times during the last 10 years, but the indications were consistently in favor of mercury compounds as affording the best protection from basal rot. With the choice of materials somewhat narrowed, a comprehensive experiment was planned to bring out significant differences, if possible on a quantitative scale, between materials having different toxic components.

Apparently healthy bulbs of the leading commercial variety, King Alfred, were selected July 28, 1938 (3 days after harvesting), from a uniform stock in which about 12 per cent of the bulbs showed basal rot when dug. After thoroughly mixing the selected bulbs, 132 random samples of 50 bulbs each were drawn. Chemical treatments with different materials, concentrations and durations were applied to different samples (Table 1), there being 4 replicate samples (200 bulbs) in each treatment. The various materials were made up in aqueous solutions or suspensions and Pyrolen M–P, a commercial wetting agent, was added to each solution at a 1 to 800 dilution.

Immediately after treatment the bulbs were placed in wire-mesh-bottomed trays, which were then stacked in the open air following a random arrangement of treatment samples in 4 replicate blocks. The stacks were supported about 1 foot above the ground and were covered with gable-roofed metal covers in accordance with commercial practice in the Long Island, N. Y., narcissus district. Two examinations for basal rot were made, the first on September 7, the second on October 2. The commercial storage period for this variety as grown in Long Island generally terminates about the middle of September. In most samples there was only a slight increase, and in many none, in the number of rotten bulbs between the two examinations. The bulbs that still appeared sound on second inspection were planted out to observe the effect of the treatment on subsequent growth and flowering.

The rot count per sample and the means for the different treatments are shown in table 1.

In the analysis of the data as here presented, the significance of the differences between treatments is postulated in relation to the error variance of the data as a whole, though it is evident that the error variance of the first 4 is less than that of the remaining treatments (actually 9.786 compared with 13.035). As the object of the experiment was a general comparison

TABLE 1.—*Effect of treating narcissus bulbs with various kinds of fungicides on the development of basal rot during storage. (The figures show the number of rotten bulbs in each unit sample of 50.)*

Chemical	Concentration	Period of immersion	Replications				Means of treatments
			1	2	3	4	
	Per cent	*Minutes*					
Mercuric oxide (HgO)	1.0	2	18	9	10	9	11.50
" chloride	0.2	5	16	10	9	11	11.50
" "	"	15	2	10	11	13	9.00
" " (acidulated[a])	"	5	8	8	9	5	7.50
" " "	"	15	10	4	7	10	7.75
New Improved Ceresan[b]	0.4	2	7	6	5	4	5.50
" " "	"	5	3	2	6	2	3.25
Cuprocide[b]	1.0	2	20	19	20	24	20.75
Coposil fungicide[b]	1.0	5	28	21	21	28	24.50
" "	"	15	22	22	21	15	20.00
Auragreen[b]	1.0	5	19	17	20	18	18.50
"	"	15	21	20	18	21	20.00
Stantex Helione Yellow[b]	1.0	5	30	18	18	18	21.00
" " "	1.0	15	15	21	19	26	20.25
Stantex Elgetol[b]	0.2	5	15	16	22	17	17.50
" "	"	15	15	12	24	17	17.00
Lime sulphur (dry)	1.0	5	19	25	17	24	21.25
" " "	"	15	18	14	18	19	17.25
Stantex Colloidal Sulphur[b]	1.0	5	21	27	19	21	22.00
Acetic acid (glacial)	1.0	5	19	8	10	10	11.75
" " "	"	15	14	11	13	14	13.00
Formalin (37 per cent HCHO)	1.0	5	17	14	20	11	15.50
" " " " "	"	15	18	14	7	15	13.50
Chlorox[b]	3.0	15	15	20	21	16	18.00
Phenol	0.5	5	21	17	18	22	19.50
"	"	15	16	10	12	16	13.50
Stantex Fungicide F–X[b]	0.2	5	21	20	22	16	19.75
" " "	"	15	18	19	19	16	18.00
Shirlan D[b]	0.4	5	16	19	21	21	19.25
" "	"	15	17	17	18	24	19.00
Silver cyanide	0.2	5	22	18	9	20	17.25
" "	......	......	25	34	25	21	26.25
Control	"	15	21	17	20	20	19.50

Difference of 4.93 between means of treatments = 5 per cent level of significance.
" " 6.54 " " " " = 1 per cent level of significance.

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[a] Contained 1 per cent acetic acid.

[b] The essential ingredients and the sources of these materials were as follows: New Improved Ceresan (5 per cent ethyl mercury phosphate)—Bayer-Semesan Co., Inc.; Cuprocide (98 per cent cuprous oxide)—Röhm & Haas Co., Inc.; Coposil (ca. 16 per cent Cu)—California Spray Chemical Corp.; Auragreen—Mallinckrodt Chemical Works; Helione Yellow, Elgetol (22 per cent sodium dinitro ortho cresylate), colloidal sulphur and Fungicide F–X—Standard Agricultural Chemicals, Inc.; Chlorox (5 per cent sodium hypochlorite); Shirlan D (salicylanilide)—Imperial Chemical Industries.

between the different chemical types, as mercury, sulphur, copper, etc., compounds, and not a close comparison between members of these groups, the segregation of error variance was not attempted. A slight obscuration of possibly significant differences between certain treatments may result, but the major conclusions as follows are not affected:

(1) A number of the treatments produced statistically significant decreases in the number of bulbs rotting in storage.

(2) Only acidulated mercury bichloride and New Improved Ceresan resulted in commercially important control of rot.

(3) The results with copper and sulphur compounds were not indicative of their prospective value for this purpose.

The New Improved Ceresan treatment for durations of 2 and 5 minutes caused both bulb and flower injury, the latter affecting about 90 per cent of the flowers produced. In all other treatments the condition of the flowers was equal to that in the checks. Since a similar treatment, applied at planting time, is an established practice in commercial narcissus culture, further work was undertaken to find means of reducing or avoiding the flower injury that results from prestorage applications.

Experience had shown that flower injury from treatment with ethyl mercury compounds preceding storage could be reduced by delaying the treatment for 1 to 2 weeks after harvesting, during which period the bulbs became appreciably more dry and firm. As previously mentioned, the reduction in phytotoxic effects was accompanied by a serious loss in efficiency of rot control, the amount of rot becoming equal to that in nontreated samples when the treatment was deferred for 2 weeks. In an effort to fix an intermediate point at which a minimum of flower injury occurs while the fungicidal benefits are preserved, a second experiment, immediately following the first, was conducted, using the same bulb stock and sampling methods. Different samples were immersed in New Improved Ceresan solution, composed of 1 lb. of chemical in 32 gal. of water, for 2, 5, or 10 minutes on the third day after digging. Similar sets of samples were treated at intervals of 6, 9, 12, and 15 days after digging. The treated bulbs were placed immediately in wire-bottomed trays to dry in the open air, and thereafter stored in 4 replicate blocks with a modified Latin square arrangement of samples. Despite their exposure in this way, the bulbs remained wet for irregular periods, and the projected 2-minute immersion was prolonged in effect so as to approximate the longer treatments. The results as regards control of basal rot are shown in table 2.

It is evident from the figures showing the number of bulbs rotting after treatment for 2, 5, or 10 minutes that the duration of treatment had but little effect on its efficiency in preventing rot. Thus the total numbers of bulbs rotting in *all* samples treated for 2, 5, and 10 minutes are respectively 306, 286, and 263, and the corresponding means are 15.3, 14.3, and 13.2. On the other hand, the total numbers of bulbs rotting in all samples treated at each of the 3-day intervals after digging are 62, 109, 205, 212, 267, showing a progressive increase in rot from the first 3-day interval to the fifth. The corresponding means are 5.17, 9.08, 17.08, 17.67, 22.25 and each difference is significant, except between the 3rd and 4th intervals. Since the duration of the treatment had no significant effect on control of rot, mean differences between the 2-, 5-, and 10-minute treatment periods were not computed but these data were combined to show the highly significant differences due to the period of delay in treating.

As before, the remaining healthy bulbs were planted out to observe the effect of the treatment on growth and flowering (Table 3). Flower injury

TABLE 2.—*The development of basal rot of narcissus bulbs in storage following treatment with New Improved Ceresan at successive intervals of 3 days between time of digging and time of treatment. All treatments in a solution of 1 lb. of chemical to 32 gal. of water*

Interval between harvest and treatment	Replicates	No. of bulbs per sample of 50 that rotted in storage after treatment for			Mean for treatment interval
		2 min.	5 min.	10 min.	
Days	*Number*				
	1	7	3	8	
	2	6	2	5	
3	3	5	6	8	
	4	4	2	6	
	Mean	5.5	3.25	6.75	5.17
	1	14	9	3	
	2	12	8	10	
6	3	6	14	7	
	4	7	11	8	
	Mean	9.75	10.5	7	9.08
	1	21	16	14	
	2	24	20	19	
9	3	11	14	19	
	4	15	15	17	
	Mean	17.75	16.25	17.25	17.08
	1	13	17	12	
	2	20	23	18	
12	3	20	18	14	
	4	21	22	14	
	Mean	18.5	20	14.5	17.67
	1	23	25	17	
	2	32	19	16	
15	3	22	18	25	
	4	23	24	23	
	Mean	25	21.5	20.25	22.25
Mean for immersion period		15.30	14.30	13.15	

Difference of 2.735 between means of treatments = odds of 19:1
“ “ 3.657 “ “ “ “ “ “ 99:1

was manifestly severe, being nearly total in the bulbs treated 3 days after digging, and marked in all the others up to the 15-day interval, when it became practically 0. Furthermore, significant differences in flower injury, in contrast to the results on rot control, appeared in the bulbs treated for different periods from 2 to 10 minutes. Thus, in the 9-day treatment interval, the 2-minute duration caused flower injury in only about 10 per cent of the bulbs as contrasted with about 68 and 95 per cent injury in the 5- and 10-minute durations. It may be seen in table 2 that the 2-minute treatment on the 9th day after digging caused a significant reduction in rot which, in

TABLE 3.—*Flower injury resulting from bulb treatments given before storage as listed in table 2*

Interval between harvest and treatment	Replicates	Flower injury after treatment for			Mean for treatment interval
		2 min.	5 min.	10 min.	
Days	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
	1	100	88	100	
	2	100	100	100	
3	3	100	100	100	
	4	74	44	79	
	Mean	93.5	83.0	94.8	90.4
	1	38	92	100	
	2	87	100	100	
6	3	90	79	96	
	4	89	100	100	
	Mean	76.0	92.8	99.0	89.3
	1	5	88	100	
	2	10	94	91	
9	3	14	68	95	
	4	15	21	100	
	Mean	11.0	67.8	96.5	58.4
	1	4	63	62	
	2	30	47	100	
12	3	24	69	84	
	4	22	33	92	
	Mean	20.0	53.0	84.5	52.5
	1	0	0	0	
	2	0	0	8	
15[a]	3	0	0	0	
	4	0	0	0	
	Mean	0	0	2	00.7
Mean for immersion period		40.1	59.3	75.4	

[a] Data from the 15-day interval were not used in the variance analysis.

Significance involving day interval

Difference of 4.489 between means of intervals = odds of 19 : 1

" " 6.036 " " " " " " 99 : 1

Significance involving periods of immersion

Difference of 3.888 between means of periods of immersion = odds of 19 : 1

" " 5.227 " " " " " " " " 99 : 1

terms of mean number of rots per sample of 50, amounts to 18 as compared with 25 for treatment on the 15th day and (from Table 1) 26 for no treatment at all.

There are circumstances when even this 31 per cent reduction in rot would be more important than the associated 11 per cent flower injury; for example, when a grower is building up a stock of valuable novelties, but for commercial purposes the control of rot would have to be improved to the level of the 3rd or 6th day treatment without the correlated increase in flower injury. This might be achieved by reducing the concentration of chemical or, in view of the previous observation that delay in natural drying of the bulbs after treatment may invalidate the regulation of the treating time, by drying the bulbs artificially.

Experiments have been undertaken employing both of these modifications. In them, as well as in commercial-scale tests by several growers in Long Island, New York, it was found possible to prevent basal rot in storage to a high degree and without encountering any visible bulb injury. The actual flowering records of the treated bulbs have not yet been obtained, and publication will, therefore, be deferred.

SUMMARY

Chemical sanitizing treatments of narcissus bulbs applied prior to the period of summer storage have been found to effect a marked reduction in basal (Fusarium) rot during the storage period.

Of the various kinds of fungicidal materials thus far tested, certain mercury compounds, particularly ethyl mercury chloride and ethyl mercury phosphate, have proved most effective. With these materials, a 2-minute immersion of the bulbs is equal to longer treatments up to 10 minutes, in protecting against rot.

Treatments applied soon after the bulbs are dug, more effectively prevent rot than do those applied later. The protective effect is practically lost in treatments delayed for more than 2 weeks.

Both of the ethyl mercury compounds cause flower-bud injury, manifest in the subsequent production of crippled flowers, when the treatments are given too soon after the bulbs are dug. This injury increases with the time of immersion and diminishes with the length of interval between digging and treating, becoming negligible after a delay of 2 weeks.

A practicable compromise between the conflicting requirements of adequate protection against rot and minimization of flower injury is foreshadowed but has not definitely been reached.

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THE CONIDIAL PHASE OF SCLEROSPORA NOBLEI

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(Accepted for publication June 23, 1941)

In 1929 the writer (11) described *Sclerospora noblei,* a new species, which had been found by R. J. Noble of the Department of Agriculture of New South Wales, occurring apparently endemic under natural conditions in Australia as a destructive parasite on *Sorghum plumosum* Beauvois (*Andropogon australis* Sprengel), a native grass locally known as "wild sorghum," of value as an abundant wild fodder for sheep and horses. At that time only the resting spore phase of this parasite was known; but as this, on comparison with other *Sclerosporas,* especially *Scl. sorghi* (Kulk.) Uppal and Weston (13), the only one whose resting spores had been reported on any sorghum previously, proved to have adequate distinguishing characters, the species was established on this basis. Naturally, however, the writer hoped that its conidial, or perhaps sporangial, phase might be discovered so that our knowledge of the species might be extended and a more adequate basis for comparison might be available. Since then Dr. Noble has found the conidial phase and this, from material that he very generously sent the writer, proves, on comparison with other species, to be distinctive also. The purpose of the following note, therefore, is to supplement the previous paper by describing the conidial phase and noting the points of interest it presents.

SEASONAL OCCURRENCE, SYMPTOMS, AND DEVELOPMENT

The wild-sorghum host is perennial and, in the cooler regions of Australia, its new growth starts in September to October, the beginning of their summer. The conidial phase of the *Sclerospora* begins to become apparent soon after and continues for some months, material being collected by Dr. Noble as late as December, January, and February.

The symptoms, like those of other systemic conidial *Sclerosporas,* involve the development, on successively unfolding leaves, of pallid, linear streaks from which, under favorable conditions, the downy growth of conidiophores and conidia emerges. Like other conidial *Sclerosporas* also, the development of the conidiophores and conidia takes place under natural field conditions during the early hours of the morning after the leaves have been covered for some time by the nocturnal condensation of dew. In the summer months in Australia Dr. Noble noted this nocturnal production of conidia between 3 and 5 a.m., not only in the field but also in clumps transplanted to the garden of his home near Sydney.

By February the ensuing oogonial or resting-spore phase is already apparent on some infected clumps, the noticeable shredding of the leaves,

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[1] Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University, No. 193.

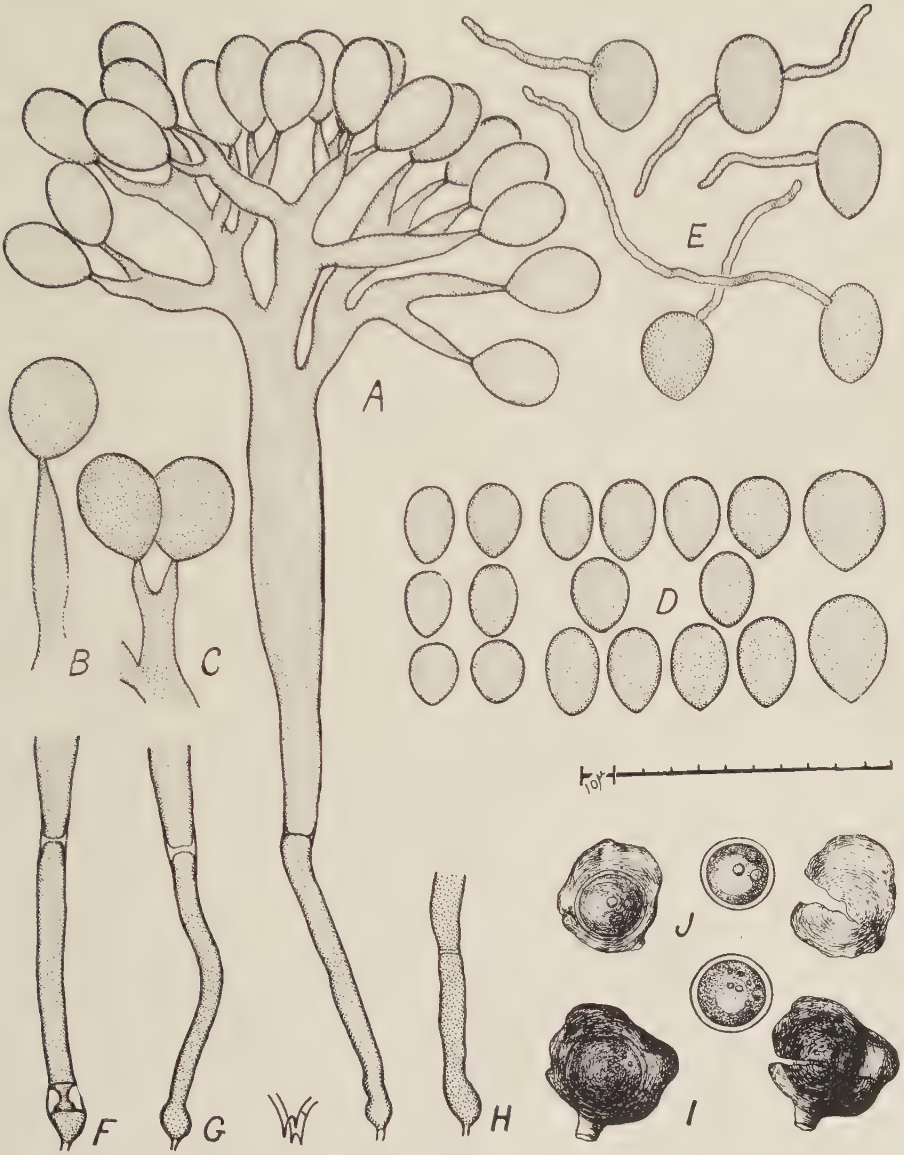

FIG. 1. Conidial phase of *Sclerospora noblei.* Camera lucida drawings from material preserved by Dr. Noble. Magnification × ± 375. *Cf.* scale. A. Typical conidiophore with bulbous footed basal cell, expanding main axis, and extensive branch system bearing numerous conidia of average size. B–C. Conidia of maximum size *in situ* showing the range in size and shape of the sterigmata from the single, long, slender branch ending of B to the short pair of C. D. Representative conidia, the middle 10 showing the predominant sizes of 25–30.9 × 20–22.9 µ, the 6 at the left the small extreme, the 2 at the right the large extreme. E. Germinating conidia showing typical germination by hyphae. F–H. Basal cells showing commonly encountered variations in size and structure. I–J. Resistant spores, at left intact showing the difficulty of discerning the characteristics of the enclosed oospore through the enveloping oogonial wall, and at right revealing these characteristics in the oospore squeezed out after treatment with acid.

so characteristic of this phase, becoming increasingly obvious throughout the remainder of the season.

CHARACTERISTICS OF THE FUNGUS

The conidiophores show the species to be allied to the destructive, predominantly conidial, *Sclerosporas* of the Orient, for they are luxuriantly developed and arbusculate, 300–450 μ in height, with a basal cell above whose septum extends the trunk-like main axis bearing the primary branches (usually 3) of approximately equal size and extent, which branch and rebranch more or less dichotomously to form an elaborate system whose ultimate tips bear the conidia (Fig. 1, A).

The basal cell, with its somewhat swollen or knobbed foot attached by a slender hypha through the stomata to the lobed clump of intramatrical hyphae in the substomatal chamber, extends upward, sometimes isodiametric, but usually expanding, its diameter from 8 to 13 μ, usually around 10 μ, its length from 68 to 110 μ most commonly about 90 μ, thus occupying about $\frac{1}{3}$ to $\frac{1}{2}$ of the complete trunk and $\frac{1}{6}$ to $\frac{1}{4}$ of the total height of the conidiophore (Fig. 1, A). The septum, which delimits the basal cell from the main axis, may be a complete cross wall (Fig. 1, G, H) or a modification of the longitudinal wall substance in the form of a ring-like thickening (Fig. 1, F) that slightly or noticeably constricts the lumen. Rarely is more than one encountered (Fig. 1, F).

The main axis expands gradually, reaching its greatest diameter of around 28 μ (20–34 μ) at a point about $\frac{1}{2}$ to $\frac{2}{3}$ its length beyond the septum, then remaining approximately isodiametric to the emergence of the branches, its length (exclusive of the basal cell) ranging from 120 to 200 μ but usually about 170 μ. The branch system is well developed and extensive, ranging from 70 to 130 μ in height and 100 to 130 μ in spread, and comprising 2 to 4 primary branches that give rise to the secondary, tertiary, or, in rare cases, quaternary sets, terminating in the tapering sterigmata, 10 to 15 μ long, that bear the conidia. The sterigmata vary in length and basal thickness, depending on whether they are the short terminations of tertiary branchlets (Fig. 1, C) or the longer endings of secondary branches (Fig. 1, B). In all cases they taper to ultimate tips of about 2 μ diameter on which the conidia are borne. As a result of the spreading branch system the conidia are disposed over a somewhat parasol-shaped or hemispherical area.

The conidia vary in number in proportion to the extent and luxuriance of the branch system, being most commonly 24 to 48, rarely more, occasionally on depauperate conidiophores with reduced branch system as few as 6 or 8.

The conidia show considerable variation in shape, but are usually obovoid with the base tapering slightly to the point of attachment and the distal end bluntly rounded, the greatest diameter being a little more than half way to the tip (Fig. 1, D).

The conidia are true conidia, the wall being thin and nearly equal throughout and absolutely lacking any thickened, modified papilla for dehiscence. In consequence, the method of germination is by hyphae, usually one, very rarely more (Fig. 1, E).

All the foregoing features, when taken together, contribute toward the characterization of the species, but no one of them in itself is particularly salient or diagnostic.

The size of the conidia, however, is a dependable characteristic, which effectually distinguishes the conidial phase of *Sclerospora noblei*. In this, as in other species of the genus, the conidial size shows considerable range, but serves as a reliable means of comparison and distinction when based on measurements of suitable numbers that express quantitatively the frequency of various size classes.

The size characteristics of the conidia of *Sclerospora noblei* are presented in table 1.

TABLE 1.—*Size measurements of the conidia of Sclerospora noblei on Andropogon australis in New South Wales, Australia. From preserved material collected by R. J. Noble*

Length		Diameter	
Classes in μ	No. of conidia in 200	Classes in μ	No. of conidia in 200
21–22.9	10	13–14.9	2
23–24.9	18	15–16.9	8
25–26.9	41	17–18.9	36
27–28.9	38	19–20.9	43
29–30.9	52	21–22.9	60
31–32.9	18	23–24.9	21
33–34.9	20	25–26.9	22
35–36.9	2	27–28.9	7
37–38.9	1	29–30.9	1

These measurements are from conidia collected by Dr. Noble during the period of optimum conidial production between 3 and 5 a.m., immediately killed in suitable fluid, and sent to the writer in preservative. The material was in excellent condition, well fixed and free from plasmolysis, but it should be noted that in the case of other species the writer, on comparing the measurements of long-preserved conidia with those of fresh conidia mounted in dew and measured immediately, has found that the preserved material usually shows a slight shrinkage.

The size frequencies as here tabulated make no pretense at being a precise statistical study, for such is not necessary to bring out the salient characteristics. It is obvious from the table that, while the length varies from the extremes of 21 to 38.9 μ, and the diameter from 13 to 30.9 μ, the range of size most frequent and representative was more restricted, 149 out of 200 being between 25–32.9 μ in length, 103 out of 200 between 19 and 22.9 μ in diameter. In size characters, therefore, they lie between the smaller, rotundspored forms such as *Sclerospora maydis* (Rac.) Palm (3) on maize in Java, with lengths most commonly between 17 and 24.9 μ, or *Scl. sorghi* (Kulk.) Weston and Uppal (13), on sorghum in India, with lengths between 19 and 24.9 μ, and the elongate-ellipsoidal-spored form *Scl. philippinensis* Weston (7) of maize in India and the Philippines, with most common lengths between 31 and 36.9 μ.

Thus *Sclerospora noblei* presents an interesting combination of characteristics; its resting-spore phase distinguished by its relatively small oospores and especially by its oogonial wall, dark, closely adherent to the enclosed oospore and unusually thick, its bluntly rounded protrusions—not outbulgings but actual thickenings to as much as 20 μ; its conidial phase distinguished by the intermediate shape and size of the conidia, which place this species between the forms with small, rotund conidia and those with large, elongate-ellipsoidal conidia. Both phases are regularly formed on the same host plant, the conidial beginning early in the growing season and lasting a few months, the oogonial appearing later and persisting until growth of the host ends for the season.

Adequate characterization of *Sclerospora noblei* now necessitates extending the diagnosis, previously based on the resting-spore phase alone, to include the distinguishing features of the recently discovered conidial stage. Also, further investigation has revealed that the previous description of the resting spore phase must be emended slightly with respect to the thickness of the oospore wall. Through the thick, dark, oogonial wall of these spores it is difficult to measure accurately the exact wall thickness of the enclosed oospore. Consequently, the writer has tried various methods for freeing the oospores of this and other species by mechanical cracking or chemical softening of the oogonial wall. Of these, treatment with acids, as suggested to the writer by Dr. E. J. Butler in 1930, has proved most practicable. When, for example, the resting spores of *Scl. noblei* are treated with concentrated nitric acid for 5 to 10 minutes, washed, and mounted, slight pressure on the cover glass will crack the oogonial walls, freeing the oospores intact (Fig. 1, I and J). These are apparently uninjured, and their diameter, 23 to 28.9 μ for 82 out of 100, agrees with that of the 600 previously measured *in situ*. Their wall thickness, however, is much greater than the 1 to 1.5 μ previously recorded, for, in 76 out of 100, this ranges from 1.75 to 3.25 μ. Further investigation is necessary to determine how much of this is actual and how much the possible effect of the acid.

Diagnosis: Sclerospora noblei.[1]

Conidiophoris 300–450 μ altis, cellula basali inferne in bulbo inflata 68–110 μ, plerumque circa 90 μ longa, 8–13 μ, plerumque circa 10 μ crassa; trunco 120–200 μ, plerumque circa 170 μ longo, leniter expanso, ad latitudinem maximum 20–34 μ, plerumque circa 28 μ, in regioni $\frac{1}{2}$–$\frac{2}{3}$ altitudinis supra septum; parte summa arboris 2–4ies dichotome ramosa, ramulis divergentibus; sterigmatibus conoideo-subulatis 10–15 μ long.

Conidiis obovoideis, 21–38 × 13–30 μ, plerumque 25–32.9 μ long., 19–22.9 μ cr., hyalinis, episporio tenui, apice sine papilla ergo semper per tubum germinantibus.

Oogoniis ovoideis vel subsphaeroideis 28–44 μ diam., membrana fusco-flavida vel castaneo-brunnea saepe fere opaca, plerumque 5–10 μ, rare 3 μ, saepe ad 20 μ, crassa.

[1] *Sclerospora noblei* sp. nov.

Oosporis sphaeroideis, 20–34 μ, plerumque 23–28.9 μ diam. hyalinis vel pallido flavidis, episporio 1–3 μ crasso, intus granulosis, guttulis oleosis praeditis. Germinatio non visa.

Hab. in foliis *Andropogonis australis* Sprengel, in N. S. Wales, Australia.

Conidiophores well developed, 300 to 450 μ in height, comprising basal cell, main axis, and spreading branch system. Basal cell with knobbed or swollen foot, then slightly expanding or more rarely isodiametric, usually about 10 μ (at times 8 to 13 μ) in diameter extending for a length of 68 to 110 μ (usually about 90 μ) to the delimiting septum, which is usually complete but may be a partial ring like thickening. Main axis expanding gradually above the septum, reaching its greatest diameter of usually about 28 μ (20 to 34 μ) at a point about ½ to ⅔ its length beyond the septum, then retaining this diameter to the first branches, its length exclusive of the basal cell 120 to 200 μ, usually about 170 μ. The branch system well developed and extensive ranging from 70 to 130 μ in height and from 100 to 130 μ in spread with 2 to 4 large primary branches, then secondary, tertiary, or rarely quaternary ones; terminating in tapering sterigmata usually about 10–15 μ long, which bear the conidia, the whole forming a subdichotomously deliquescent branch system so arranged that the conidia are spread over a parasol-shaped or hemispherical area.

Conidia obovoid ranging from 21 to 38.9 μ by 13 to 30.9 μ, most frequently 25 to 32.9 μ by 19 to 22.9 μ. Conidia hyaline with thin, continuous, unmodified wall and granular content, germinating invariably by hyphae.

Resting spores (as previously described) showing rather obviously that they comprise a modified oogonium with oospore within. Oogonium usually ovoid, ellipsoid, pyriform or subspherical in shape, occasionally with bluntly rounded projections rendering it gibbous and unsymmetrical, ranging in diameter from 28 to 44 μ. Oogonial wall closely adherent to the oospore within, the exterior protrusions not representing outbulgings, but rather involving actual increases in thickness of the wall, which most commonly is 5 to 10 μ, at times only 3 μ, often as much as 20 μ. Wall dark, often scarcely transparent, color ranging from somewhat golden (Mars yellow Ridg.) to rich-brown (Brussels brown Ridg.), most commonly dark resinous amber (Sudan brown Ridg.). Fragment of oogonial stalk frequently adherent. Oospores regularly spherical, most frequently from 23 to 28.9 μ in diameter, the mode between 25 to 26.9 μ, extremes ranging from 20 to 34 μ; wall hyaline to pale golden, 1 to 3 μ thick; content finely granular with aggregations of denser material and with masses of oily reserve substance, central to eccentric in position.

Germination of the resting spores not yet observed.

Both phases occurring on *Sorghum plumosum* Beauv. (*Andropogon australis* Sprengel) in New South Wales, Australia, collected by R. J. Noble. The conidial phase occurring on pallid streaks on the leaves in summer (Dec., Jan., Feb.), developing as a downy outgrowth at night when the leaves are wet with dew; the successive oogonial phase beginning in Jan. and Feb. and continuing to develop throughout the growing season, causing the disintegration of the interfascicular tissue and the fraying of the leaves into withered tangled shreds.

DISCUSSION

Now that our knowledge of *Sclerospora noblei* has been amplified by the discovery and study of the conidial phase, further points of interest merit consideration.

The relationship of this species is now clarified, its conidia with finality precluding any alliance with the sporangial (potentially zoospore producing) *Sclerospora graminicola,* and definitely indicating association with the series of species characterized by true conidia. In this series the size and shape of its conidia place *Scl. noblei* in an intermediate position between the group with relatively small, rotund conidia (*e.g., Scl. maydis* and *Scl. sorghi*) and the group with larger, more elongate-ellipsoidal conidia (*e.g., Scl. philippinensis* and *Scl. sacchari* [1]).

Moreover, *Scl. noblei* regularly develops both its conidial and its resting-spore phase successively on *Andropogon australis,* a point of resemblance to

Sclerospora sorghi, which forms both phases on *Sorghum vulgare,* but a distinct contrast to *Scl. maydis, Scl. philippinensis* and *Scl. spontanea* (8), which, on maize, an introduced host, form only the prominent conidial phase, but are suspected of developing both phases on wild grasses within their geographic range, and a point of disagreement with such species as *Scl. northi* on *Erianthus* in the Fiji Islands (10) and *Scl. butleri* on *Eragrostis* in Nyasaland (12), in which the resting spore phase seems the predominant one or at least the only one as yet known.

Furthermore, *Sclerospora noblei* is distinctive and specifically characteristic in both its conidial and its resting-spore phases, and can be identified with certainty in either. This distinctive individuality of both phases is in marked contrast to the situation in *Scl. sorghi* and *Scl. graminicola,* which, although absolutely distinct from each other in their respective conidial or sporangial phases, are very difficult to distinguish in their resting-spore stages.

In the second place, in the development of its conidial phase *Sclerospora noblei* corroborates the correlation of conidiophore production in *Sclerospora* under natural field conditions with the nocturnal suffusion of the leaf surface with dew or other moisture, a correlation first formulated by the writer (9) in the case of *Scl. philippinensis* and *Scl. spontanea,* and since confirmed in the case of other species by various investigators, most recently by Steyaert (4).

Finally, *Sclerospora noblei,* since its discovery by Dr. Noble ten years ago, has been found only on the one original host, *Andropogon australis* (2). During this time no evidence of its transfer to other hosts in the field has ever been encountered nor was Uppal (13) in his cross-inoculation studies with *Scl. graminicola, Scl. sorghi,* and *Scl. noblei* able to secure infection by *Scl. noblei* on *Andropogon sorghum, Pennisetum typhoideum, Setaria italica, Euchlaena mexicana,* and 3 varieties of *Zea mays,* even though he used the same technique of inoculation by resting spores in the soil that has proved notably successful in the case of other species. Possibly after the vicissitudes of their journey from Australia to India the resting spores may not have been germinable; in any case, such negative results in a relatively small number of tests can be regarded only as indicative rather than conclusive.

Yet, despite the foregoing evidence, it does not seem safe to the writer to dismiss *Sclerospora noblei* as of no potential menace to valuable cultivated gramineous crops, for the cumulative evidence from successful cross inoculations with other species (*cf.* 5, 6) seems to justify regarding any *Sclerospora* occurring on members of the Andropogoneae as possibly dangerous to cultivated Gramineae. Indeed, this species must now be considered as potentially even more dangerous than before, because its conidial phase, although not producing the vast quantities typical of *Scl. philippinensis* on maize (9), develops formidable numbers of infective conidia in successive crops for months during the growing season.

SUMMARY

The writer's original characterization of *Sclerospora noblei* on its distinctive resting-spore phase is here supplemented by the description of its equally distinctive conidial stage more recently collected by Dr. Noble. The well developed, arbusculate conidiophores and the true conidia ally this species with the predominantly conidial species of the Orient; the size of the conidia, most commonly from 25 to 32.9 μ by 19 to 22 μ, together with the relatively small oospores and dark, unusually thick oogonial wall previously noted, distinguishing this species.

The species is compared with others and such points of interest are noted as the regular occurrence of both reproductive phases on the same host, the development of the conidiophores at night when the plants are wet with dew, and the apparent limitation of this parasite to its indigenous Australian grass host.

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VARIETAL RESISTANCE TO BLOSSOM–END ROT IN TOMATOES[1]

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Physiological blossom-end rot has caused large losses each year in spring tomatoes (*Lycopersicon esculentum* Mill.) in eastern Texas. Severe loss from blossom-end rot in most commercial fields of tomatoes in 1937 apparently resulted from abundant rains, promoting succulent growth of the plants, followed by unusually long periods of drought. Despite certain drought periods in the 3 succeeding years, rains were abundant and distributed well enough to promote large yields of fruit; blossom-end rot caused relatively little loss in most of the fields of Marglobe and Rutgers tomatoes.

REVIEW OF LITERATURE

Brooks (1) reported blossom-end rot resulting from both drought and excess water. He described blackened placentae near the blossom-end of fruit with normal external appearance. Stuckey (5) found that resistance of tomatoes to blossom-end rot is inherited. Higgins (3) stated that blossom-end rot of pepper is caused by drought following a period of rapid growth of plants and fruit. Under such conditions, wilting leaves may remove water from cells in the blossom-end of fruits. More recently, Foster (2) found that high-nitrogen plants were very susceptible to blossom-end rot, whereas applications of superphosphate decreased the amount of this abnormality. Wedgworth (6) also presented data showing the relative amounts of blossom-end rot in several varieties of tomatoes; and the writer (7) classified recent commercial varieties as resistant or susceptible to this trouble.

MATERIALS AND METHODS

In this work, the fields in which the plants were set were plowed in November, and 600 lb. per A. of a 6–10–7 fertilizer were distributed in the rows late in March. Tomato seeds treated with red or yellow cuprous oxide were planted in hot beds about February 10, and the seedlings were transplanted 4 in. apart in cold frames about March 5. Early in April, when the plants were 6 to 12 in. tall, they were transplanted from cold frame to field, usually in units of 24 plants each. The plants were set 21 in. apart in rows 5 ft. apart. Each plant was pruned to one main stem and tied to a stake. The fields were cultivated frequently with sweep blades, from April to June, to control weeds and to keep the soil crust broken. All plants received a side dressing of 200 lb. 6–10–7 fertilizer per acre about May 7 each year, except for differences in side dressing fertilizer, as noted elsewhere. Fruits with blossom-end rot were picked and recorded every 7 to 10 days.

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[1] Approved as Technical Contribution No. 760 by the Director of the Texas Agricultural Experiment Station.

TABLE 1.—*Amount of blossom-end rot in tomato varieties, 1937 to 1939, inclusive*

Variety	No. of plants	Blossom-end rot; number of fruits per plant			Variety	No. of plants	Blossom-end rot; number of fruits per plant		
		Min.	Max.	Ave.			Min.	Max.	Ave.
Baltimore	517	0.2	2.1[c]	0.9	Louisiana Pink[b]	1082	0.2	2.2	0.9
Bay State	81	0.1			Louisiana Red[b]	1183	0.5	3.6	1.6
Blair Forcing[b]	530	0.0	0.2	0.1	Marglobe[b]	3503	0.0	1.3	0.2
Break O'Day[b]	603	0.1	0.7	0.4	Marhio[b]	320	0.1	0.7	0.3
Brimmer	63	0.7	......	......	Marvana[b]	373	0.2	2.3	0.7
Brown's Special	90	0.8	3.4	2.0	Marvel[b]	214	0.2	1.0	0.5
Buckeye State[b]	317	0.7	6.1	1.9	Marvelosa	204	0.6	3.9	1.8
Century[a]	402	0.2	2.6	0.9	Michigan State[b]	528	0.1	0.8	0.5
Columbia	229	0.3	2.2	0.8	New Invincible	188	0.1	1.0	......
Duke of York	166	0.1	......	......	Newport 4 and 5[b]	396	0.2	1.0	0.4
Early Baltimore[b]	259	0.0	1.0	0.4	Norton	137	0.2	0.7	
Everbearing Scarlet Globe	72	0.2	......	......	Norduke	81	0.4	......	......
Extra Early Prolific	138	1.0			Oxheart	66	0.3	0.7	
Garraprihe[a]	104	0.0	1.3	0.2	Pearson	92	0.1	0.3	
Globe (Livingston, Landreth)	301	0.2	0.4	0.3	Prairiana[b]	349	0.5	2.9	2.1
Globelle 1 and 2	159	2.4	3.9	3.1	Pritchard[b]	794	0.0	0.6	0.2
Globelle 3	70	1.0	......	......	Red Cherry	66	0.0		
Glovel	112	1.5	2.0	......	Redfield Beauty	36	0.6	......	......
Golden Queen	92	......	1.1	......	Riverside[a]	331	2.0	5.0	3.6
Grothen's Red Globe[b]	392	0.2	1.3	0.6	Rutgers[b]	2467	0.1	4.0	0.9
Gulf State Market[b]	958	0.2	3.8	1.1	Summerset	207	0.0	1.2	0.4
Illinois Baltimore[a]	471	0.2	2.5	0.9	Sureset Forcing[b]	362	0.2	0.9	0.5
Illinois Pride[b]	233	0.3	2.9	0.6	Sweetmeat[a]	151	0.3	2.8	1.6
Indiana Baltimore	64	0.7	2.4	......	Tennessee Red[a]	83	0.2	......	......
Invincible	172	0.7	1.6	......	Urbana Forcing	69	0.3	......	......
Kanora[a]	155	0.1	1.0	0.9	"White-flowered" selection	801	0.0	0.5	0.3
Lloyd Forcing	90	0.1	......	......	Yellow Ponderosa	60	0.1	0.3	......
Long Calyx Forcing[b]	289	0.0	0.3	0.1					
Louisiana Dixie[a]	144	0.3	4.7	2.8					
Louisiana Gulf State	45	0.3	......	......					

[a] Variety tested 2 years. [b] Variety tested 3 years. [c] Variation in numbers of fruits with blossom-end rot per plant was due mainly to variation in fields and to combining many selections of varieties in summarizing data.

The plants were sprayed with calcium arsenate and copper fungicides to control insects and diseases attacking the leaves and fruit.

The data in table 1 were obtained from plants growing in Ironton red soil that was abundantly infested with the tomato-wilt fungus, *Fusarium lycopersici* Sacc., but it was practically free from the root-knot nematode, *Heterodera marioni* (Cornu) Goodey (8). Consequently, blossom-end-rot data were recorded only for the varieties that showed time-weighted wilt resistance of at least 40 per cent. Wilt-susceptible varieties, including Bonny Best and Stone, were omitted from the table because the plants died of wilt before sufficient information was secured as to their susceptibility to blossom-end rot.

EXPERIMENTAL RESULTS

As recorded in table 1, the following varieties showed the most resistance to blossom-end rot: Blair Forcing, Break O'Day, Grothen's Red Globe, Long Calyx Forcing, Marglobe, Marhio, Marvana, Marvel, Michigan State, Newport, Pritchard, Surest Forcing, and "White-flowered" (9) selections. The resistance of the Pritchard, Marglobe, and "White-flowered" selections was evident also when the plants were grown in soil practically free from *Fusarium lycopersici.* Thus, fusarium wilt showed no apparent effect on the resistance of the tomato varieties to blossom-end rot described in table 1. This may be explained by the fact that plants with moderate or severe symptoms of fusarium wilt may suffer from inadequate water, even when growing in moist soil. Foster (2) has stated that plants growing continuously with low soil moisture appear to be resistant to blossom-end rot.

Comparative data on blossom-end rot in different varieties were secured also from tomatoes grown in fields free from soil-inhabiting parasites. Amounts of blossom-end rot were calculated separately for groups of rows of Marglobe (Landreth Certified) tomatoes without sprays and for groups of rows separately sprayed with 8 copper sprays each year. The plants were grown in fields of Norfolk fine sandy loam soil, practically free from *Fusarium lycopersici* and *Heterodera marioni.* In the 4-year period, the

TABLE 2.—*Relation of blossom-end rot to yield of tomatoes—1940*

Tomato variety	No. of plants	Number of blossom-end-rot fruits per plant	Tons of marketable fruit per acre
Buckeye State	129	5.0[a]	0.879
Grothen's Red Globe	122	6.7	6.825
Gulf States Market	132	7.6	7.694
Illinois Baltimore	132	7.1	6.497
Louisiana Red	128	17.2	5.688
Marglobe	127	2.9	8.339
Pritchard	125	1.2	8.762
Rutgers	133	7.1	6.898
"White-flower" selection	135	2.1	7.717

[a] Before June 5th, Buckeye State variety bore too few fruits to show its susceptibility to blossom-end rot.

groups of plants showed 0.1 to 1.7 fruits with blossom-end rot per plant. There was no correlation between the amount of blossom-end rot and differences in the copper-spray formulae. The Marglobe tomatoes regularly showed strong resistance to blossom-end rot, with only a narrow range in the amounts of the disease in the different years. This range was similar to that for Marglobe selections described in table 1.

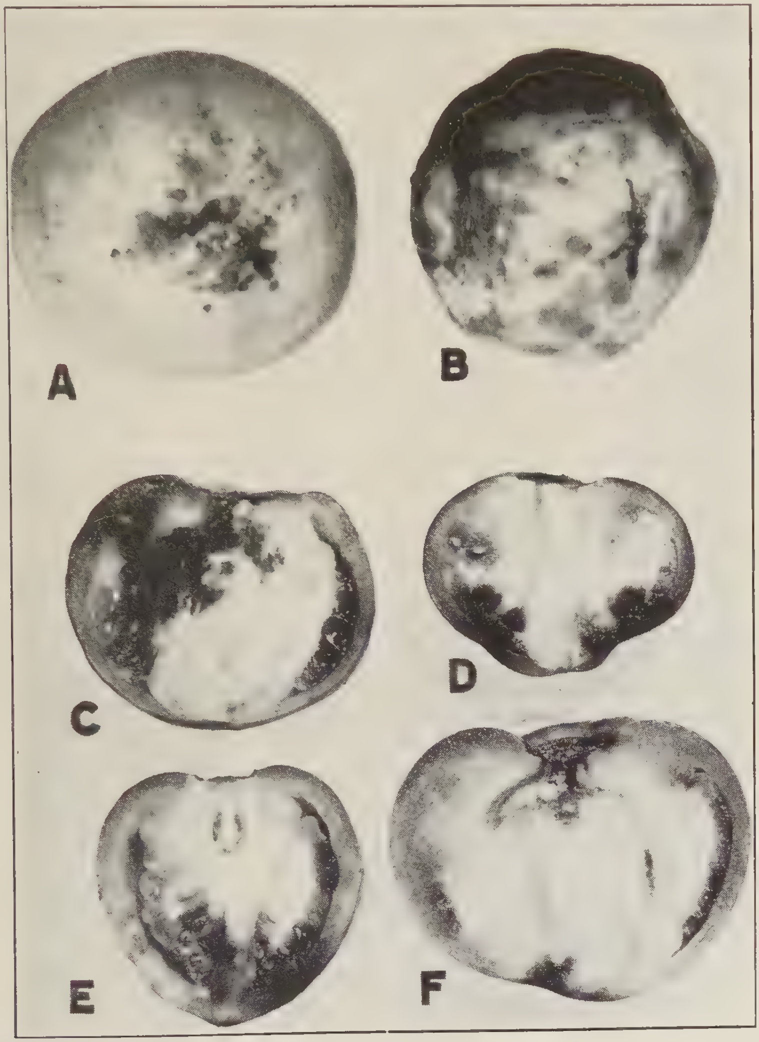

FIG. 1. Symptoms of blossom-end rot of tomatoes. A. Ordinary early externally visible symptoms consisting of brown or black blotches in peel. The following unusual symptoms are illustrated: B, brown depressions indicating deep necrotic tissues in fruit; C, D, E, necrotic areas in fruits showing superficial brown depressions; F, vascular browning near stem end associated with blossom-end rot, and possibly related to physiological core rot.

Table 2 compares the amounts of blossom-end rot and yields of fruit of 9 varieties of tomato, each growing in 6 randomized plots of Norfolk fine sandy loam, practically free from soil-borne parasites. The plants were set on April 4, 1940, and the records on blossom-end rot were first taken on May 25. The disease was more severe in Marglobe tomatoes growing in this part of the field with very light sandy soil receiving 10–0–10 fertilizer in the side dressing, than in the adjacent part with heavier sandy soil and 6–12–6 fertilizer in the side dressing. The difference may be due to the lower amount of phosphate and the lower water-holding capacity of the soil in the former part of the field. Although blossom-end rot decreases tomato yields, the lower yields were not closely correlated with the numbers of diseased fruits per plant. Such a correlation apparently is affected by differences in the fruit-setting tendencies of the different varieties.

Also, in 1940, 100 plants of Buckeye State tomato, in a field of Ruston soil, averaged 3.9 fruits with blossom-end rot per plant with the same fertilizer treatment as that described for the plants in table 2. In the other experimental fields, the many varieties averaged less than 0.1 fruit with blossom-end rot per plant during this same year.

The first externally visible symptom of blossom-end rot in eastern Texas usually appears as light-brown or abnormally dark-green, apparently water-soaked spots, $\frac{1}{16}$ to $\frac{1}{2}$ in. wide in the peel of the blossom-end of tomato fruits $\frac{1}{2}$ to 3 in. in diameter. At first, the spots are limited to the subepidermal cells (Fig. 1, A). Later, the necrotic lesion enlarges until it involves deep tissues and the spot becomes a sunken, brown area with a distinct margin. Many of the small fruits become hemispherical or more extremely flattened from shrinkage of the lower part of the blossom-end-rotted fruit, a condition especially noticeable in the fruit of Louisiana Red and Buckeye State varieties. Areas with blossom-end rot often are rotted by species of *Alternaria* and *Fusarium*. In addition to these symptoms, an unusual type of the disease appeared during rainy weather in June, mainly in Buckeye State tomatoes. These fruits had uneven surfaces with brown areas, indicating internal brown tissues (Fig. 1, B–E).

The number of fruits with blossom-end rot per plant gives only an estimate of decreases in calculated yields, because varieties of tomatoes differ much in the number of fruits formed, and in the percentage of their fruits that develop the disease while they are very small. Estimates are useful, however; so the following method of calculation was tried in 1939: Six bushels of green-wrap tomatoes contained 1370 individual fruits with an average weight of 3.35 oz. each. With the usual 4978 plants per acre, loss of one blossom-end-rotted fruit per plant gave a calculated loss of $\frac{1}{2}$ ton of fruit per acre. Blossom-end rot rarely causes greater loss of Marglobe tomatoes. Using a variety with ordinary susceptibility, *e.g.*, Rutgers, 143 plants produced an average of 4 large end-rotted fruits per plant, or a calculated loss of 2 tons of fruit per acre. A like loss was seen in 5 acres of the same variety in which blossom-end rot ruined more than half of the large yield of fruit.

Resistance to blossom-end rot may be stronger in one strain of tomato than in another strain of the same variety. For example, T708 Rutgers was more resistant than T638 Rutgers, and T50 Marvelosa was more resistant than T57 Marvelosa.

CONTROL

Blossom-end rot is a physiological abnormality, attributable probably to a varietal weakness. Accordingly, the relative resistance to blossom-end rot may be related to the extent and efficiency of the root systems of different varieties of tomatoes. Although there is a certain amount of variation in the resistance to blossom-end rot in a given variety or selection in different fields and from year to year, yet the range in such variation indicates that some varieties are resistant, while others are moderately or extremely susceptible.

Fall tomatoes in the Winter Garden region of Texas are grown in fertile soil. With the usual adequate irrigation, the plants suffer little from drought. The typically large vines cover the ground and develop fruit in October when the weather becomes cool. Surveys in that locality in 1936, 1937, and 1939 showed that blossom-end rot was rare, even in the susceptible Rutgers and other varieties of tomato. This is evidence that the rot may be controlled by irrigating tomatoes enough to avoid drought injury and facilitate abundant yield of fruit. Stuckey and Temple (4) found less of the disease following irrigation.

Practical control of blossom-end rot of tomatoes without irrigation, in eastern Texas, consists mainly of planting the varieties Marglobe and Pritchard, using 600 to 800 lb. of 6–10–7 or 6–12–6 fertilizer per acre, and cultivating the plants to give them the most uniform, favorable water supply available each year. Even when the soil is moist, a day or more of warm, dry wind may cause some drought rigor in tomatoes. Shallow cultivation presumably delays the evaporation of water from wet soil, increases the oxygen available to the roots, makes the plants depend on deep roots in moist soil, and conserves water and fertilizer for the tomatoes by controlling the weeds. Tomato plants should be cultivated every 7 to 10 days, especially as soon as a soil crust forms after each rain or rainy period until most of the crop has been picked. The lightest sandy fields should be avoided, as they may favor the development of blossom-end rot.

SUMMARY

Marglobe and Pritchard are the commercial varieties strongly resistant to blossom-end rot in eastern Texas. The tests showed least blossom-rot in Blair Forcing, Break O'Day, Grothen's Red Globe, Long Calyx Forcing, Marglobe, Marhio, Marvana, Marvel, Michigan State, Newport, Pritchard, Surest Forcing, and "White-flowered" selections. Most of the other varieties tested were moderately susceptible to the disease. Riverside, Louisiana Red and Buckeye State were extremely susceptible.

Fusarium wilt had no apparent effect on the resistance of tomatoes to blossom-end rot.

An uncommon symptom of blossom-end rot is described.

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HOST RANGE AND GROWTH–TEMPERATURE RELATIONS OF CORYNEUM BEIJERINCKII[1]

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The shot-hole disease of *Prunus* spp., caused by *Coryneum beijerinckii* Oud., has a wide geographical distribution. It is found in France, Italy, Germany, and probably in other parts of Europe, and has been reported from Asia, Argentina, Algeria, Australia, New Zealand, Mexico, and the United States.

On peaches, in California, the disease has been observed[2] to cause a shot-hole effect on the leaves and to kill the young growth, but it is not found in equal severity in all the fruit-growing districts of the State. It is reported of rare occurrence in Santa Clara County and has never been observed in Alameda County.

The host range of *Coryneum beijerinckii,* as reported by Wilson,[3] includes the following species of stone fruits: *Prunus armeniaca* L., *P. avium* L., *P. communis* Fritsch (*P. amygdalus* Stokes), *P. davidiana* Franch, *P. domestica* L., *P. laurocerasus* L., *P. padus* L., *P. persica* Sieb. and Zucc., *P. persica* var. *nectarina* Maxim., *P. serotina* Ehrh., *P. virginiana* L.

Blodgett[4] lists *Prunus emarginata* Walp. as susceptible to *Coryneum beijerinckii* in Idaho. Flachs[5] reports that *P. laurocerasus,* the cherry-laurel, is occasionally attacked in Germany by an organism closely related to *C. beijerinckii* and states that reddish-yellow to brownish spots are found on the leaves, the tissue thus spotted eventually falling out and leaving a shot-hole effect.

In southern California during the 1940–41 season, the rainfall, which occurred mostly in March and April, was approximately twice the normal amount, and conditions were especially favorable for the development of *Coryneum* on early-leafing varieties of stone fruits. The shot-hole disease was observed in plantings at the Citrus Experiment Station on leaves of *Prunus fenzliana* Fritsch (S.P.I. 27336), *P. majestica* Koehne (S.P.I. 55417), and *P. umbellata* Ell., and in commercial orchards on leaves of almond, apricot, Japanese plum (very severe), and peach. Inoculation studies for the determination of host range and temperature studies of the disease undertaken at that time are herein reported.

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[1] Paper no. 447, University of California Citrus Experiment Station, Riverside, California.

[2] Smith, Ralph E., E. H. Smith, T. F. Hunt, and B. J. Jones. California peach blight. California Agr. Exp. Stat. Bull. 191: 73–98. 1907.

[3] Wilson, Edward E. The shot-hole disease of stone-fruit trees. California Agr. Exp. Stat. Bull. 608: 40 p. 1937.

[4] Blodgett, Earle. Fruit diseases reported from Idaho. U. S. Dept. Agr. Bur. Plant Ind. Pl. Dis. Rptr. 21: 215. [Mimeo.] 1937.

[5] Flachs. Schrotschusskrankheit an Kirschlorbeer. Blumen und Pflanzenbau 42: 65. 1927.

ARTIFICIAL INOCULATIONS

Artificial inoculations with *Coryneum beijerinckii* were made on different species of *Prunus.* The cultures used in the inoculations were isolated from peach, apricot, and almond. These isolates appeared to be indistinguishable as to character, and were readily cross-inoculable between different species of *Prunus.*

Spores of *Coryneum beijerinckii* were atomized onto the new growth of leaves and stems. While still wet, the stems were punctured by means of a steel needle. (The leaves were not punctured.) Some inoculations did not require protection to maintain proper moisture conditions for development of the infection; others were enclosed for 48 hours in a moist chamber or in a cellophane sack. So far as is known, no previous reports of comparative inoculations with spores of *C. beijerinckii* on *Prunus* have been made.

About 1 to 2 weeks after inoculation, typical spots appeared on the leaves. The diseased tissue soon dropped away and left the typical shot holes. The following species of *Prunus* were found to be susceptible to inoculation with *Coryneum beijerinckii: P. alleghaniensis* Porter,*[6] *P. angustifolia* Marsh,* *P. armeniaca* L., *P. armeniaca* var. *mandshurica* Maxim.,* *P. avium* L., *P. besseyi* Bailey, *P. bokhariensis* Royle (S.P.I. 40223), *P. caroliniana* Ait. (*Laurocerasus caroliniana* Roem.), *P. cerasifera* Ehrh.,* *P. davidiana* Franch (S.P.I. 36664), *P. demissa* Walp. (*P. virginiana* var. *demissa* Torr.), *P. domestica* var. *insititia* Bailey,* *P. fenzliana* Fritsch (*Amygdalus fenzliana* [Fritsch] Korsh.) (S.P.I. 27336), *P. fremontii* Wats., *P. hortulana* Bailey,* *P. ilicifolia* Walp., *P. kansuensis* (*Amygdalus kansuensis* [Rehder]) (S.P.I. 68976), *P. lyonii* Sarg., *P. mahaleb* L., *P. majestica* Koehne (S.P.I. 55417), *P. maritima* Marsh,* *P. mexicana* Wats., *P. mume* Sieb. and Zucc., *P. munsoniana* Wight and Hedr.,* *P. orthosepala* Koehne,* *P. persica* Sieb. and Zucc., *P. pissardii* Koehne, *P. pseudo-cerasus* Lindl. (S.P.I. 18587), *P. reverchonii* Sarg.,* *P. salicina* Lindl., *P. serotina* Ehrh., *P. spinosa* L., *P. texana* Dietr., *P. umbellata* Ell. (S.P.I. 38974), *P. watsonii* Sarg., *P. yedoensis* Mats.,* *Prunus* spp. (S.P.I. 31652, 33217, 56121) Tunisian almond (S.P.I. 101096).

Lesions on stems caused by inoculations through puncture wounds developed on *Prunus armeniaca, P. kansuensis, P. persica, P. mahaleb, P. mira* Koehne (S.P.I. 34601), *P. munsoniana* (lesions coalesced with gum formation), *P. salicina,* and *P. yedoensis.*

While as noted by Wilson,[7] moisture is an important factor in the development of *Coryneum beijerinckii* on host plants, age of the leaves is also important. Young, succulent leaf tissue proved more favorable for spore infection than older leaf tissue. This fact was observed in many of the inoculations, especially those on apricot leaves and on leaves of *Prunus lyonii* and *P. ilicifolia.*

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[6] The species marked with an asterisk (*) were secured from the Arnold Arboretum as seed or scions.

[7] Wilson, Edward E. The shot-hole disease of stone-fruit trees. California Agr. Exp. Stat. Bull. 608: 40 p. 1937.

Leaf infections of 3 evergreen species of *Prunus, P. ilicifolia, P. lyonii,* and *P. caroliniana,* are shown in figure 1. These species are closely allied with *P. laurocerasus.* On *P. lyonii* (Fig. 1, F) there was one large infected area that did not fall out, and several smaller ones. The same was true of

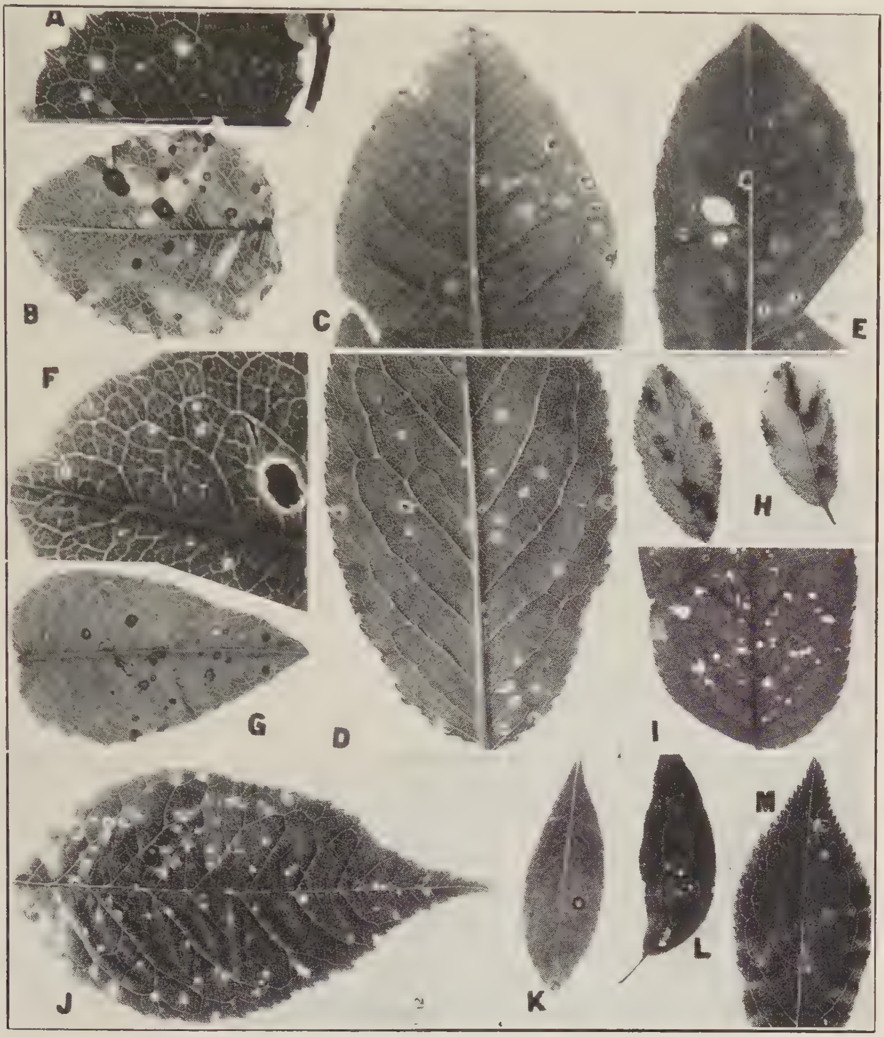

FIG. 1. Leaves of *Prunus* spp. artificially inoculated by atomizing the uninjured leaves with spores from cultures of *Coryneum beijerinckii* isolated from *Prunus* spp. A. *P. ilicifolia* (from almond) after 15 days. B. *P. ilicifolia* (from apricot) after 20 days. C. *P. demissa* (from peach) after 8 days. D. *P. orthosepala* (from *P. caroliniana*) after 6 days. E. *P. caroliniana* (from apricot) after 25 days. F, G. *P. lyonii* (from almond): F, after 45 days; G, after 6 days. H. *P. texana* (from apricot) after 14 days. I. *P. mexicana* (from peach) after 13 days. J. *P. hortulana* (from almond) after 12 days. K. *P. besseyi* (from apricot) after 19 days. L. *P. reverchonii* (from apricot) after 19 days. M. *P. maritima* (from peach) after 12 days.

infected areas on *P. ilicifolia.* Lesions were produced on leaves of *P. caroliniana* by infection through natural injuries, as well as through the stomata. Several of the infected areas dropped out, and the resulting shot-hole effect may be seen in figure 1, E. These 3 species and the other species of *Prunus* illustrated in figure 1, *P. besseyi, P. demissa, P. hortulana, P. maritima, P. mexicana, P. orthosepala, P. reverchonii,* and *P. texana,* are all native to the United States.

The different species of *Prunus* do not appear to be equally susceptible to *Coryneum beijerinckii,* but the data on this subject are not sufficient for comparison of susceptibility. The results of inoculation of *P. domestica, P. salacina,* and *P. serotina* showed only a few spots and spot holes. *P. besseyi* developed a few brownish spots 2 to 3 mm. in diameter. *P. hortulana, P. reverchonii,* and *P. umbellata* are extremely susceptible.

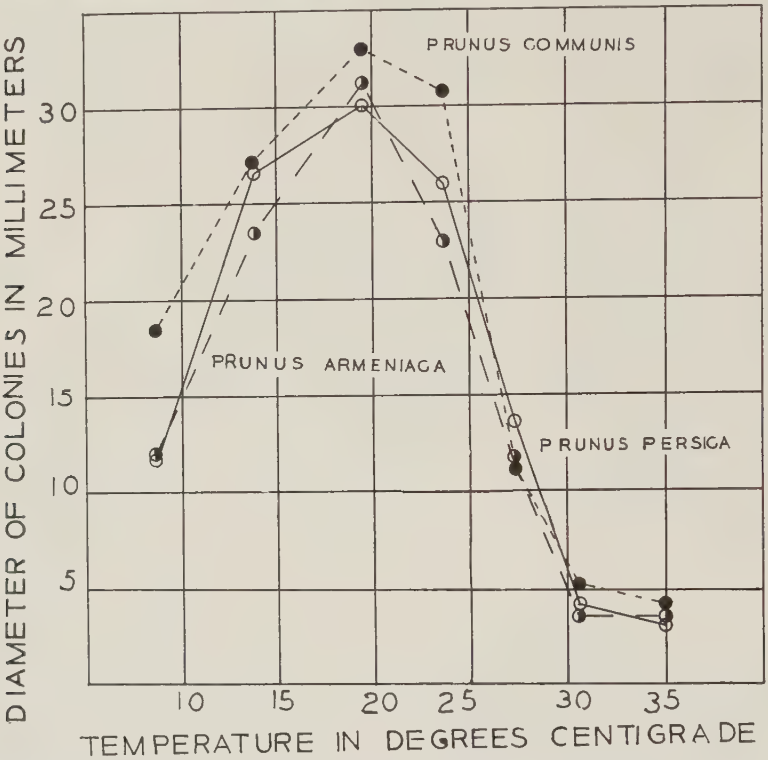

FIG. 2. Curves showing growth-temperature relations of *Coryneum beijerinckii* isolated from apricot (*Prunus armeniaca*), peach (*P. persica*), and almond (*P. communis*), when grown at constant temperatures. Optimum mycelial growth occurred at about 19° C.

From the positive results obtained in the inoculation tests, it appears that the organism is omnivorous and that other species of *Prunus* will be susceptible under favorable conditions.

GROWTH-TEMPERATURE RELATIONS

Temperature is believed to be an important factor in the development of *Coryneum beijerinckii.* The disease appears to develop best at temperatures prevalent during cool rainy weather of winter and spring in California.

Growth from pure cultures of the 3 isolates (from apricot, peach, and almond) was tested at constant temperatures of 8, 13, 19, 23, 27, 30.5, and 35 degrees C. for a period of 1 week, and measurements of the diameters of the colony were made every 2 days. The curves (Fig. 2) are similar for each of the isolations: with increase in temperatures, the growth curves became much steeper; optimum growth occurred at about 19°. Growth was considerably reduced at 27°, and none occurred at 30.5° C. (Fig. 2 and 3).

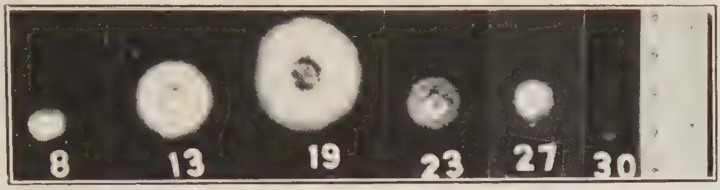

FIG. 3. Relative size of colonies of *Coryneum beijerinckii* from apricot, after 7 days' growth on glucose-potato agar at the temperature indicated (°C.).

Spore formation was observed in the colonies at the lower temperatures. A few spores were formed at 8° and 13°, and there was abundant spore formation at 19° and 23°. No spores were observed at the higher temperatures of 27° and 35° C.

SUMMARY

Inoculations with *Coryneum beijerinckii* on leaves and stems of *Prunus* spp. gave positive results of infection on about 35 species. The disease was indicated by a shot-hole effect on the leaves, although spots that did not fall out sometimes appeared in the leaf tissue. The spots appeared on the uninjured leaves in 1 to 2 weeks after inoculation.

From inoculation tests so far, it seems apparent that the organism is omnivorous in attacking species of *Prunus*. Among the species showing positive results were *P. ilicifolia, P. lyonii,* and *P. caroliniana,* all evergreen species under California conditions. *P. demissa,* the western choke cherry, was also susceptible.

Optimum development of the organism in culture tests occurs at about 19° C. A few spores are produced at 8° and 13°. Abundant sporulation occurs at 19° and 23°; no spores were observed at temperatures of 27° and higher.

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MEASURING MAGNITUDE OF A DEFOLIATION DISEASE OF TOMATOES[1]

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INTRODUCTION

One of the difficult problems in plant pathology is to measure rapidly and precisely the magnitude of disease attack. Stevens (6) has recently made an earnest plea for recording disease attack, even though quantitative methods for making such measurements may not be available.

While making a quantitative study of the performance of protective fungicides on tomato foliage, it became imperative to make first a quantitative study of magnitude of infection. Otherwise, the effect of the fungicide on infection could not be measured with precision.

When dealing with a large number of field plots, it is necessary to have a rapid method that is also precise. McKinney (5) has proposed a rapid technique that depends upon grouping plants into 5 classes based on magnitude of disease. This method is less objective than desirable because it depends somewhat upon judgment. Likewise, its precision was not known. The objectiveness and precision of the method have been investigated.

MATERIALS AND METHODS

The defoliation disease of tomatoes caused by *Alternaria solani* was used as the test disease. The comparative data were obtained from a series of plots sprayed with various copper compounds where the amount of disease varied with the performance of the materials.

Counting Disease

Counting leaf spots is highly recommended, because of its objectivity, as a method of measuring magnitude of infection, but it is seldom used because it is so slow that not enough plants can be examined in an experimental plot to offset the errors due to plant variability. The objectivity is somewhat less than perfect, also, if more than one disease is prevalent or if insect punctures are present.

The number of counts can be reduced without sacrificing much objectivity if leaves are divided into 2 groups—healthy and sick, as Martin (4) has done in his research on tomato fungicides. Even this is much too slow. The fewest counts, and, therefore, the fastest method, is to divide plants into 2 groups—sick and healthy. This method has a low order of precision, however, because the number of plants required for precision at both ends of the scale cannot be provided in any reasonably-sized experimental plot.

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[1] The research for this paper was begun at the New York State Agricultural Experiment Station, Geneva, N. Y., and completed at the Connecticut Agricultural Experiment Station, New Haven, Conn. It was conducted in cooperation with the Crop Protection Institute.

The McKinney Grouping Method

The McKinney (5) method is essentially a compromise in this case. As applied here to a defoliation disease, it has the rapidity of the plant-counting technique because the condition of individual plants is recorded. It has most of the precision of the leaf counting technique because each plant is ranked into 1 of 5 groups on the basis of the leaf area attacked. It loses a certain amount of objectivity, however, because the individual leaves are not examined. The amount of disease on the leaves is estimated.

The infection ratings are based, therefore, on the relative proportion of total leaf area on the individual plant killed by fungus attack. The infection categories are as follows: 0 = infection-free or nearly so, 1 = trace to 25 per cent of leaf area killed, 2 = 26 to 50 per cent killed, 3 = 51 to 75 per cent killed, 4 = 76 to 100 per cent killed. In practice 2 men should record data on any series of experimental plots. One man walks crosswise of the plots, so that they lose their identity. He calls out the category number of each plant. This is recorded by the second man on a plot map. The data are calculated by the following formula:

$$\text{Infection index} = \frac{\Sigma \text{ category numbers}}{\text{No. plants} \times 4} \times 100$$

The 4 in the denominator represents maximum infection and 100 is used to convert to percentage. In dealing with fungicides the infection index is subtracted from 100 to give percentage control, which brings the data into line with other toxicological data.

Marsh, Martin, and Munson (3) state that the grouping procedure is open to statistical analysis on the basis that ''although the estimates are not necessarily in direct linear relation to the amount of fungus present . . . , they are reducible to a linear function of this amount.''

The chief drawback to the method is that it is difficult to assign the same meanings to the infection categories from time to time. This, however, is not particularly troublesome to the trained observer.

Experience has shown that McKinney's 5 groups make a useful number. Marsh, Martin, and Munson (3) used 10 for a project on potato fungicides; but it seems to the writers that use of 10 groups adds more confusion by doubling the number of border-line possibilities than it adds in precision. With 5 groups the dividing lines are so far apart that errors in judgment on border-line cases are insignificant.

Yield

Yield records are usually considered close approximations to objective records, and they have found some favor as a measure of magnitude of disease. Fungicides are often compared through yield, but, since fungicides often dwarf plants, their protective value cannot always be compared through yield.

In the case of vegetables that, like tomatoes, are picked fresh, yield records are particularly difficult to assess because of the difficulty in assigning a valid criterion of ripeness. Fruits on defoliated tomato vines usually come up to an orange color, seldom to the deep red that fruits attain on non-defoliated plants. Obviously, both do not come to the same stage of ripeness. As will be shown elsewhere (2), relation between disease and yield of ripe tomato fruit may be negative, absent, or positive depending upon many factors.

EXPERIMENTAL

The fungicide experiments at Geneva, N. Y., in 1938, offered an opportunity for comparing several methods of recording magnitude of infection because *Alternaria solani* attacked so frequently that the untreated plants were almost completely defoliated by mid-September. The treatments differed sufficiently among themselves to give a spread in magnitude of infection over almost the whole range.

For each treatment there were 4 replicate field plots of 20 plants each of a pure line of John Baer tomato. There were 12 treatments and a check.

The amount of stem-end rot was recorded on the ripe fruits as they were picked during the season. At the end of the season in late September, the following data were recorded (Table 1): infection index, as just described, green weight of fruit, green weight of vine less fruit, number of dead, sick, and healthy leaves per plant (5 plants only per plot). The data have been studied by graphic comparison.

TABLE 1.—*A comparison of various methods of measuring incidence of defoliation on tomatoes caused by Alternaria solani at Geneva, N. Y. in 1938*

Treatment	Disease index	Wgt. of green fruit	Wgt. per green plant less fruit	Leaves killed per plant	Stem-end rot on fruit
	Per cent	*Pounds*	*Pounds*	*Per cent*	*Per cent*
A	34.8	5.06	2.41	29.5	14.7
B	47.2	5.41	2.40	30.1	22.0
C	50.2	5.81	2.43	30.4	24.1
D	53.0	4.84	2.39	33.8	26.7
E	58.8	5.01	2.30	36.4	25.0
F	61.8	4.71	2.07	39.3	32.6
G	62.8	3.88	2.06	41.3	34.9
H	65.0	4.22	2.00	42.1	43.5
I	65.2	4.25	2.03	47.0	39.9
J	71.6	3.84	1.89	48.1	35.2
K	86.6	2.36	1.43	58.7	44.5
L	99.4	1.88	1.07	66.4	51.5
Check	99.6	2.17	1.18	68.7	48.1

Relation between Infection as Judged and as Counted

The relation is linear between the infection index obtained by the subjective judging technique and the percentage of leaves killed or the percentage of fruits invaded by stem-end rot, obtained by the more objective counting technique (Fig. 1, A and B). When the curves are extrapolated, they intersect the abscissa at the zero point in each case. It is clear that the judgment method of recording magnitude of infection is not only valid and, therefore, sufficiently objective within this experiment, but it also is precise.

It is noteworthy that the curve relating killed leaves to infection index reaches the ceiling of infection index at about 65 per cent of killed leaves.

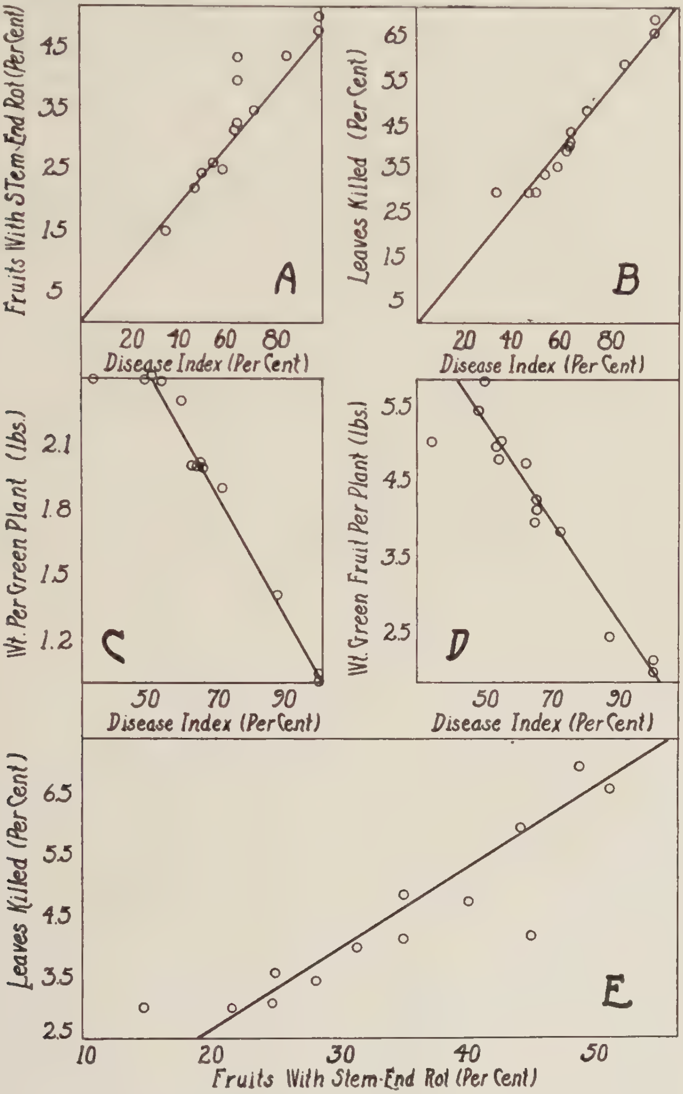

FIG. 1. A graphic comparison of methods of measuring magnitude of infection by *Alternaria solani* on tomatoes. A. Relation between infection index and percentage of fruits affected by stem-end rot. B. Relation between infection index and percentage of leaves killed. C. Relation between infection index and weight of green plants less fruit. D. Relation between infection index and weight of green fruits at the end of the season. E. Relation between percentage of fruits affected by stem-end rot and percentage of leaves killed.

The reason for this seems clear. The visual readings are based on the *area* of leaves, whereas the counting system is based on the *number* of leaves that have been killed. In the counting system a relatively resistant tiny new leaf, just unfolded at the apex of the stem, carries as much weight as a big susceptible leaf at the base, so that not more than 65 per cent of the total number of leaves may have been killed on plants that look almost denuded of effective foliage. Stated otherwise these data emphasize clearly that counting the number of leaves killed does not give as true a picture of the number of successes registered by the fungus as an estimate of the leaf area destroyed, despite the fact that the former is a more nearly objective method than the latter.

Since both the percentage of infection on leaves and percentage of infection on fruit bear a linear relation to infection index, it follows that the relation between the two should be linear. A simple plotting of the data (Fig. 1, E) shows that such is the case.

Relation between Infection Index and Weight of Green Fruit and Green Plant

The weight of green fruit and the weight of the green plant less fruit at the end of the season can be measured objectively. They should be closely correlated inversely with the infection index because the tissues not directly affected by disease are present for weighing. It was of interest, therefore, to observe a linear relation between disease index and weight of green fruit and weight of green plants less fruits (Fig. 1, C and D). Here it is also clear that the grouping technique gives valid and precise data.

Agreement between Workers

During 1940 a similar experiment was made on Scarlet Dawn tomatoes at New Haven, Conn., comparing 7 copper materials with a check, on 4 replicate plots of 10 plants each, making a total of 280 plants. On September 7, when the disease was becoming serious, the plots were rated independently by each of 3 workers after a preliminary conference on the groupings. Data (Table 2) show that every material, with a single exception, was rated in precisely the same order by all 3 readers, even when the materials differed by only 2 or 3 per cent. The single exception is materials D and E, where D was rated ½ per cent better and 1 per cent better than E by readers

TABLE 2.—*Readings of disease index on plots of tomatoes treated with different fungicides to control Alternaria solani*

Treatment	Reader No. 1	Reader No. 2	Reader No. 3
A	62.5	66.5	66.2
B	63.0	69.0	68.3
C	76.0	73.5	77.0
D	79.0	78.5	79.5
E	80.5	79.5	79.0
F	87.5	86.5	84.0
G	90.5	87.5	87.5
H	99.5	98.5	98.0

No. 1 and No. 2 respectively, but it was rated ½ per cent poorer by reader No. 3.

Usefulness for Dosage-control Curves

There is one last bit of interesting evidence of the precision of the technique. Recently, it has been shown that dosage can be related in a linear fashion to disease control (1) and that data obtained with the McKinney (5) technique fit the curves smoothly.

DISCUSSION

The precision of the McKinney infection-index technique is most striking, as shown by the linear relations to the counted percentage defoliation, percentage of stem-end rot, green weight of vine less fruit, and green weight of fruit. The agreement for most of the points is so close that any point off the curve is worthy of further investigation as an aberrant case, not as throwing doubt upon the validity of the agreement. In the case of weight of green plant less fruit the only point off the curve is the first one. This shows lower green weight than expected. This point is for Bordeaux mixture, which is already known to dwarf tomatoes.

The technique is somewhat less than completely satisfactory for comparing one year with the next, because it is difficult to keep the standards unchanged. This would also be a difficulty in comparing records at one research station with those at another. Nevertheless, for one investigator and especially for one series of plots, the technique is well adapted.

It is probable, however, that any judgments are influenced by the general level reached in any particular season. If most of the plants are infected, a mediocre plant may pass for excellent, but if most plants are not infected a mediocre plant may pass for poor.

This difficulty can be reduced by preparing charts as standards, such as those used by the cereal-rust investigators.

SUMMARY

The McKinney rapid technique for measuring the magnitude of infection of a plant disease has been applied to tomato defoliation.

Each plant is examined by walking crosswise of the plots and assigned an arbitrary number from 0 to 4, ranging from no disease to complete defoliation. The ratings are summed, divided by the total number of plants multiplied by 4, since 4 is the highest possible amount of disease. The quotient is multiplied by 100 to convert to percentage, which is called index of infection.

This rapid, if somewhat subjective, method of measuring infection was found to bear a linear relation to the following approximately objective methods of reading infection on the same plots: (1) percentage of leaves killed by the fungus, (2) percentage of fruits invaded by stem-end rot, (3) weight of green plants less fruits at time of reading, and (4) weight of green

fruits at the end of the season. Using this rapid technique 3 men read 8 spray treatments and rated them in the same order of effectiveness.

From these facts it follows that the method is valid, precise, and sufficiently objective.

Although the method is precise for reading a single series of fields or plots, it has the loophole that the standards are difficult to maintain precisely from season to season.

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ISOLATION AND INFECTION TESTS WITH SEED- AND SOIL-BORNE COTTON PATHOGENS[1]

W. W. RAY AND J. H. MCLAUGHLIN

(Accepted for publication July 7, 1941)

Seedling diseases of cotton are important to the grower because of poor stands, the necessity of replanting, stunted plants, decrease in production because of skips in rows, excess amount of seed required, the many seedling diseases that later develop into lint- and boll-destroying diseases, the lessened value and amount of lint cotton produced per unit area, and the weakening of plants, making it much easier for secondary organisms to infect the cotton.

Damping-off and other seedling diseases of cotton plants may be caused and accelerated by a number of organisms. Arndt (1) isolated the following organisms from diseased seedlings: *Glomerella gossypii, Fusarium* spp., *Rhizoctonia solani, Pythium ultimum,* and several species of bacteria. Of these organisms, *G. gossypii* caused the most damping-off, whereas the various species of *Fusarium* produced lesions on roots and hypocotyls, but not damping-off. *Rhizoctonia solani,* isolated in but 10 per cent of the attempts, was not considered important as a cause of damping-off in cotton. Lehman (4) tested 35 seed lots of the 1936 cotton crop from 19 counties in North Carolina. He found 40 per cent of the seed in 31 of the lots infested with *G. gossypii* and 31.4 per cent of the seed of all lots infested with various species of *Fusarium* among which *F. moniliforme* was frequently recognized. Camp *et al.* (2) report from a survey in Florida the diseases most common during the first two months of the season to be sore shin, caused by several organisms, particularly *Rhizoctonia solani;* angular leaf spot (*Bacterium malvacearum*); and wilt (*Fusarium vasinfectum*). They also reported the presence of a large proportion of *F. moniliforme.*

Miller and Weindling (6, 7) and Weindling *et al.* (8) have shown that *Glomerella gossypii* is rarely found in Oklahoma and Texas, but that it is the fungus most often isolated from diseased seedlings in the Southeastern States. Recent surveys of our own substantiate their data with reference to the scarcity of *G. gossypii* in Oklahoma, for it has been found in but few counties in the eastern part of the State.

In many respects the organisms isolated from diseased seedlings in Oklahoma are similar to those reported by Miller and Weindling in the past few years for the other cotton-belt States.

Weindling *et al* (8) have shown that *Fusarium moniliforme* is more often associated with diseased seedlings than is any other fungus in Oklahoma. Our data have shown this to be true. Although it has been shown by Wood-

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Acknowledgments are made to Dr. K. S. Chester for helpful suggestions on the experiments and preparation of the manuscript and to Dr. C. D. Sherbakoff for his aid in the identification of the Fusaria.

roof (9) that this fungus is pathogenic, our tests have shown that it is not so severe in its attack as *G. gossypii, Rhizoctonia solani,* and a few other species of *Fusarium.* But because of its great frequency in Oklahoma soils, this fungus must be considered an important cause of seedling diseases of cotton.

Rhizoctonia solani is the second most commonly isolated fungus from seedlings in Oklahoma. There is no doubt about its importance as a cotton pathogen.

Since *Glomerella gossypii* is of minor importance in Oklahoma, a condition contrary to that east of this State, an attempt has been made to classify the organisms causing seedling diseases on the basis of their relative importance in this State. Also it was thought necessary to identify the various species of *Fusarium* and to establish their importance, if any, as pathogens. To accomplish these aims, it was first our purpose to collect samples of diseased seedlings throughout Oklahoma and to isolate and identify the organisms present. Samples have been taken for a period of 2 years, and more will be taken in the future. A second purpose of this study was to test the pathogenicity of the organisms (Table 2) under various environmental conditions and to rank the organisms on the basis of these tests according to their importance as cotton pathogens.

TABLE 1.—*Tabulation of the more pathogenic fungi isolated from cotton seedlings in 21 counties of Oklahoma in 1939*

Name of the organism	No. of times isolated	Percentage of fungi isolated
Fusarium moniliforme	61	40.00
F. scirpi	8	3.90
F. vasinfectum	5	2.40
F. scirpi v. *acuminatum*	2	0.90
F. solani	21	10.30
F. solani v. *martii*	20	9.90
F. equiseti v. *bullatum*	1	0.49
Rhizoctonia solani	40	19.80
Sclerotium bataticola	24	11.80

FUNGI ASSOCIATED WITH DISEASED COTTON SEEDLINGS

In table 1 is presented a list of the more pathogenic fungi with the percentage of times they were isolated from field samples taken in 21 counties of Oklahoma in 1939. This list does not include such commonly isolated genera as *Chaetomium, Rhizopus, Aspergillus, Alternaria, Penicillium,* and some sterile forms, for tests have shown that such fungi are either saprophytes or weak parasites.

FUNGI ASSOCIATED WITH COTTON SEEDS AND BOLLS

Numerous isolations of fungi have been made from seed, particularly from their interiors, and fewer have been made from boll lesions. A tabulation of the organisms isolated from seedlings, seeds, and bolls is given in table 2.

TABLE 2.—*A list of fungi isolated from seedlings, seeds, and bolls of the cotton plant*

Name of the organism	Isolated from		
	Seedlings	Seeds	Bolls
Glomerella gossypii (South.) Edg.	+	+	+
Fusarium moniliforme Sheld.	+	+	+
F. vasinfectum Atk.	+	−	−
F. scirpi Lamb and Fautr.	+	+	+
F. solani (Mart.) App. and Wr.	+	+	+
F. semitectum Berk. and Rav.	−	+	+
F. chlamydosporum Wr. and Rg.	−	+	−
F. scirpi L. and F. v. *acuminatum* (E. and E.) Wr.	+	+	+
F. equiseti (Cda.) Sacc. v. *bullatum* (Sherb.) Wr.	+	+	−
Rhizoctonia solani Kühn	+	−	−
Sclerotium bataticola Taub.	+	−	−
Alternaria spp.	+	+	+
Penicillium spp.	+	−	+
Aspergillus spp.	+	−	+

PATHOGENICITY TESTS OF THE MORE IMPORTANT COTTON FUNGI

Materials and Methods

The cotton seed used in these experiments consisted of 3 varieties, namely, Acala, from the 1938 Oklahoma crop, Paymaster, from the 1939 Texas crop, and D. and P. L. 11A, from the Mississippi crop of 1938.

Experiments were conducted to test the pathogenicity of the various organisms by: (a) inoculation of the organisms into sterile soil and subsequent planting of sterile cotton seed, and (b) inoculating cotton seedlings grown under sterile conditions in test tubes on water agar.

The seeds were delinted with concentrated sulphuric acid and graded as suggested by Chester (3), and only the heavy, fungus-free fraction was used. These were then surface-sterilized by immersing in a calcium hypochlorite solution containing 2.79 per cent of available chlorine.

Five cultures of each organism were grown on agar slants, and at the end of 10 days the contents of the tubes were removed and ground in a small amount of sterile sand. This was then added to enough sterilized soil[2] to fill twenty 2¼-in. pots. Three seeds were planted in each pot and kept moist with sterile water. Data were obtained at the end of 28 days in most tests.

It was not possible to control the environmental factors within definite, small limits, but the environmental differences between the various experiments were diverse enough to give a distinct contrast. Rather than tabulate the lengthy data of these experiments, which are somewhat preliminary in nature, only a summary will be given with special reference to those organisms possessing the highest degrees of virulence.

EXPERIMENTS

1. *Standard Conditions.* With the average temperature of the greenhouse at 80° F. and with ample water for the plants, the 4 most virulent

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[2] The soil for these experiments consisted of 2 parts loam, 1 part sand, and 1 part sewer sludge.

organisms of those tested were, in order: *Glomerella gossypii, Rhizoctonia solani, Fusarium scirpi* and *F. moniliforme.* Some of the other fungi produced minor lesions on the roots and hypocotyl, but no serious damage was done to the seedlings.

2. *Excessive Moisture.* When the plants were watered heavily several times a day in the 80-degree greenhouse, all of the organisms tested showed an increase in virulence with the exception of *Fusarium vasinfectum,* which decreased. *F. moniliforme* displayed a decided increase in pathogenicity.

3. *Deficient Moisture.* The soil was watered just enough to keep the plants growing. *Glomerella gossypii, Rhizoctonia solani,* and *Fusarium scirpi* were highly pathogenic, whereas the other fungi tested decreased in their virulence.

4. *Low Temperature.* The plants were held for several days at 80° F. and then placed outdoors, where the temperature averaged near 65° F. Both *Glomerella gossypii* and *Rhizoctonia solani* were very pathogenic and caused damping-off. The other organisms in the test did no serious injury to the plants.

5. *Alkaline Soil.* Enough lime was added to the soil to give a reaction of pH 8.3. *Glomerella gossypii* and *Rhizoctonia solani* were the only fungi of those tested that were virulent, and they caused considerable damping-off. The other fungi did no noticeable damage to the plants.

6. *Acid Soil.* Enough sulphur was added to the soil to give a reaction of pH 6.3. *Rhizoctonia solani,* followed closely by *Glomerella gossypii,* was the most pathogenic of the organisms tested. The remainder of the fungi produced but slight infections with no prominent injury to the plants.

DISCUSSION

Since the results of these various experiments are not directly comparable, it seemed desirable to arrange a classification of the organisms involved according to their relative degrees of pathogenicity. In these tests 3 degrees of injury to the plants were recognized: *slight,* no evidence of injury observable, yet root systems visibly attacked; *moderate,* root systems obviously attacked but with only slight injurious effect upon the plants; and *heavy,* root systems and plants displayed serious injury or death. Arbitrarily, heavy infection was given a value of 3 points, moderate infection 2 points, and slight infection 1 point. An index of infection for each organism in each experiment was calculated by multiplying the percentage of plants heavily infected by 3, moderately infected by 2, and slightly infected by 1, totaling, and averaging.

Since *Glomerella gossypii* proved in these experiments to have the highest index of infection, it was given an arbitrary weighted index of 100. The indices of infection of all the other fungi were weighted in proportion to the arbitrary weighted index of infection assigned to *G. gossypii.* The weighted indices of the organisms employed in the various experiments is indicated in table 3.

TABLE 3.—*The weighted indices of infection of the fungi used in the soil culture experiments. The letters and numbers refer to the identity of the isolate used in the experiments*

Name of the organism	W. I.	Name of the organism	W. I.
Glomerella gossypii	100	*Sclerotium bataticola* (41)	45
Rhizoctonia solani	86	*Fusarium semitectum*	42
Fusarium scirpi (D)	58	*F. moniliforme* (115)	40
F. scirpi v. *acuminatum*	56	*F. vasinfectum* (24)	37
F. chlamydosporum	53	*F. moniliforme* (18)	35
F. moniliforme (110)	52	*F. vasinfectum* (27)	34
F. vasinfectum (27)	48	*Sclerotium bataticola* (R37)	30
F. scirpi (44A)	47	*Aspergillus* sp.	29
F. solani	46	*Penicillium* sp.	28
F. equiseti v. *bullatum*	46		

The pathogenicity of the various fungi was determined also by the inoculation of seedlings grown in large test tubes on water agar. Using the "weighted index of infection" method, and applying it to the test tube experiments, a definite correlation with the results obtained in the pot cultures was established. In general, it was found that the weighted indices were higher than for the pot cultures, but in the same relative order.

Although field tests have not been attempted, it is predicted that the infection indices will be lower due to a lower inoculum potential and competition from other soil organisms.

CONCLUSIONS

Glomerella gossypii, despite its high degree of pathogenicity, is probably of little importance in Oklahoma because of its infrequent occurrence.

Apparently strains varying in pathogenicity exist in *Fusarium moniliforme, F. vasinfectum, F. scirpi,* and *Sclerotium bataticola,* as evidenced by the weighted indices of infection of the several isolates of these species.

Sclerotium bataticola (*Rhizoctonia bataticola* (Taub.) Butler), although common in Oklahoma soils, is of slight consequence as a pathogen of cotton seedlings.

Fusarium moniliforme, although it does not possess a high degree of virulence, might conceivably become, because of its high frequency of occurrence, a serious cotton-seedling parasite under favorable conditions.

The majority of the other species of *Fusarium* and those of *Alternaria, Penicillium, Chaetomium, Aspergillus,* and *Rhizopus* are not considered important parasites of cotton seedlings in Oklahoma, either because of their lack of virulence or because of their scarcity in the soil.

Rhizoctonia solani, because of its high degree of virulence under various environmental conditions, and because of its frequency in Oklahoma soils, is considered to be the most important fungus involved in diseases of cotton seedlings. The real importance of this fungus may not be indicated by the actual percentage of isolation for several reasons. Tests in our greenhouse and those by Lehman (5) show that seedlings cannot be protected satisfactorily against this organism by the seed treatments thus far employed. We have observed here that Ceresan-dusted seed, planted in soil heavily infested with this fungus, often do not emerge at all. It is conceivable that the long skips in rows so commonly observed in the fields throughout the State may be due largely to a high inoculum potential in the soil. In such a situation no samples from which to make isolations are taken.

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A PHYSIOLOGICAL STUDY OF THE SUSCEPTIBILITY OF THE BLUSHED AND GREEN SIDES OF APPLES TO CERTAIN FUNGOUS ROTS[1]

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INTRODUCTION

As the season advances, most apple fruits gradually develop a blush on the side facing the sun. Brooks and Fisher (1926) found that the osmotic concentration of the apple sap was higher on the blushed side than on the green side. Haynes and Archbold (5) report higher sugar content on the blushed side of maturing apple fruits. Other internal differences also were thought to exist. The present study was undertaken to test the difference in susceptibility of the blushed and green sides of apple to certain fungous rots and its relation to certain physical and chemical properties of the two sides.

MATERIALS AND METHODS

Two tests were made on July 22 and August 1, 1940. In the first test 4 varieties of apple, Wealthy, McIntosh, Cortland, and Snow, were inoculated with 4 fungi, namely, *Sclerotinia fructicola* (Wint.) Rehm., *Penicillium expansum* Link, *Physalospora malorum* (Peck) Shear, and *Lambertella corni-maris* von Höhnel. In the second test one other variety of apple, Duchess, was added but only 3 of the organisms were employed, *P. malorum* being omitted. Along with the inoculation tests internal factors such as water content, total nitrogen content, total ácidity, sugar content, and tissue firmness were determined.

Inoculation. Inoculations were made on the blushed and green sides of 16 fruits of each variety of apple with each fungus. A pyramidal-shape block of tissue was cut out from the side of each apple and into the cavity thus formed a small rectangular piece of mycelium, cut out from the margin of an actively growing Petri-dish culture, was inserted. The block of tissue was then replaced without sealing. Care was used in cutting the blocks of apple tissue and the pieces of mycelium for inocula to ensure uniformity in all inoculations. The inoculated apples were put in paper bags and kept in moist chambers consisting of wooden apple boxes lined with pliofilm and equipped with tight covers.

Measurement. The diameter of the rotted area of the fruit was measured 3 days after inoculation with *Sclerotinia fructicola,* 5 days after inoculation with *Penicillium expansum,* 6 days after inoculation with *Physalospora*

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[2] The writer wishes to acknowledge his indebtedness to Doctors D. G. Clark, A. B. Burrell, and H. H. Whetzel for their constructive criticisms and suggestions during the course of the present study, to Dr. F. M. Blodgett for his help in statistical analyses of the data and to Mrs. F. Hayes for reading the manuscript.

malorum, and 10 days after inoculation with *Lambertella corni-maris*. The method of measurement consisted in slicing off a portion of the apple tissue at the bottom of the inoculation cavity through the line XY, as shown in figure 1, A, and measuring the diameter (the distance CD, Fig. 1, A) of the rotted area thus exposed. In case the decay was too far advanced, as is shown in figure 1, B, a slice of the tissue was first cut off through the line XY, and then another slice was cut through the lines BM and BN, respectively, on either side. The sum of the lines AB and BC was taken as the diameter of the rotted area.

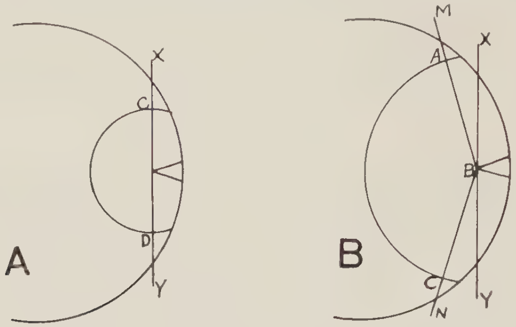

FIG. 1. A and B illustrate points made in the text concerning methods of measuring the rotted area of apple fruits.

Determination of the Internal Factors. Apples for such determinations were picked from the same trees as those from which fruits were picked for inoculation. Duplicate composite samples were prepared from 20 apples of each variety for the determination of water content, nitrogen content, total acidity, and sugar content.

For the determination of water content, the apple was pared, and 3 longitudinal slices of approximately equal size were cut from the fruit and placed in a Petri dish that had been lined with filter paper and weighed. The Petri dish was kept covered so that an excessive loss of moisture from the sample might be avoided. The sample was quickly weighed, the total fresh weight of each sample being 35 to 42 grams. It was then dried in a ventilated Freas electric oven at 60° C. for about 72 hours and again weighed.

The dried sample, together with the filter paper from the moisture determination, was digested, and the total nitrogen content was determined by Gunning's modification of the Kjeldahl method.

For the acidity determination the apples were likewise pared, and several longitudinal slices were cut from each of the 20 apples and placed in a wide-mouth bottle. The composite sample was frozen and thawed. The juice was pressed out with a metal hand press and centrifuged for 4 minutes. A 10-cc. sample of the supernatant liquid was pipetted out and placed in a 150-cc. Erlenmeyer flask. About 20 cc. of distilled water was added. The juice was titrated against N/10 NaOH solution using phenolphthalein as an indicator. A satisfactory absolute end-point was difficult to get, but a comparative end-point was easily obtained when the pinkish shadow of the dark-colored juice was cast upon a white table and compared with that of the first sample, as a lamp shone through the two samples from an equal distance of about 8 inches.

For the determination of sugar content the fruit was pared, and a small tangential section was sliced off, and then small cubes about 1 mm. across were cut and placed directly in a 150-cc. beaker kept in a moist chamber. The composite sample, averaging about 40 g., was quickly weighed and dumped into 50 cc. of boiling, 95 per cent alcohol in a wide-mouth bottle. Heating was continued for 15 minutes so as to stop enzyme action. The sample was later extracted in a Soxhlet extractor, with about 100 cc. more of 95 per cent alcohol, for 5 hours. The extract was evaporated down to 10 cc. 3 times and then diluted with 20 cc. of warm distilled water, cleared, deleaded, made up to volume, and analyzed for reducing and total sugars by the official gravimetric method.

For the determination of tissue firmness, 20 apples of each variety were tested with a Magness pressure tester. A small portion of the skin was sliced off, and the pressure tester was then applied to the exposed cut surface of the flesh. The firmness was measured by the pounds of pressure required to puncture the tissue.

RESULTS

The results of the tests are presented in table 1 and discussed below.

Difference in Susceptibility of the Two Sides of Apple

Sclerotinia fructicola. With this fungus the rate of rotting was invariably faster on the green than on the blushed side, and the difference was statistically significant in 7 out of 9 cases. When all the data of both inoculation tests were analyzed together, the odds were found to be over 9999 : 1, the decay being more rapid on the green side.

Penicillium expansum. In the first inoculation with *P. expansum* the green side of 3 out of 4 varieties of apple rotted faster than the blushed side, but only in the variety Wealthy is the difference statistically significant. In the second inoculation, the fungus invaded 4 of the 5 varieties significantly faster on the green than on the blushed side; in the other variety the rate of rotting was about equal on the two sides. Analysis of the data of the two inoculations together showed significantly more rapid rotting of the green side.

Physalospora malorum. The rate of rotting on the blushed side of two apple varieties was faster than that on the green side in the test with this fungus; in the other two varieties the order was reversed, and the difference was small. When the data on the 4 varieties were analyzed together, the difference was insignificant.

Lambertella corni-maris. Analysis of the data as a whole showed no significant difference between the two sides in rate of rotting by this fungus.

TABLE 1.—*The relation of the rate of rotting of apple to certain physical and chemical properties of the blushed and green sides of the fruit*

Date	Variety	Side	Diameter of rotted area (mm.)				Water (per cent fresh weight)	Nitrogen (per cent fresh weight)	Total acidity[b]	Sugar (per cent fresh weight)			Tissue firmness (pounds pressure to puncture)
			Physalospora malorum (6 days)	*Lambertella corni-maris* (10 days)	*Penicillium expansum* (5 days)	*Sclerotinia fructicola* (3 days)				Total	reducing	sucrose	
Aug. 22	Wealthy	Blushed	18.1[a]	25.1	18.5[a]	22.3[a]	88.2	0.029	13.64	....	....	....	24.2[a]
		Green	16.1	24.8	20.4	24.4	90.3	0.025	12.84	....	....	....	21.9
	McIntosh	Blushed	15.9	24.4	12.7	30.1[a]	85.5	0.037	9.84	....	....	....	23.0[a]
		Green	16.4	24.8	12.9	33.1	87.5	0.034	10.18	....	....	....	20.5
	Cortland	Blushed	16.6[a]	24.1[a]	14.1	26.0[a]	85.5	0.047	7.17	....	....	....	23.7[a]
		Green	14.7	22.8	15.1	28.4	87.3	0.042	6.55	....	....	....	22.1
	Snow	Blushed	16.7	26.8[a]	16.9	22.1[a]	85.6	0.027	11.61	....	....	....	28.4[a]
		Green	17.4	28.2	16.6	23.8	86.5	0.026	11.33	....	....	....	26.6
Sept. 1	Wealthy	Blushed	....	12.5	22.4	25.3[a]	88.2	0.028	11.88	8.59	7.58	1.01	18.1[a]
		Green	....	12.9	22.3	26.9	89.7	0.027	11.75	7.13	6.42	0.71	17.2
	McIntosh	Blushed	....	13.2	18.2[a]	21.2[a]	85.1	0.029	13.58	9.71	8.21	1.50	22.3[a]
		Green	....	13.2	19.3	23.4	86.9	0.029	13.64	8.39	7.58	0.81	20.0
	Cortland	Blushed	....	17.6	18.3[a]	26.2[a]	85.3	0.041	7.39	9.42	7.87	1.55	23.1[a]
		Green	....	17.1	19.9	28.3	87.0	0.042	6.69	8.55	6.88	1.67	22.1
	Snow	Blushed	....	19.8	19.9[a]	24.5	85.6	0.026	11.47	8.60	7.20	1.40	26.2[a]
		Green	....	19.1	20.6	24.8	86.5	0.026	12.13	7.97	6.69	1.28	25.0
	Duchess	Blushed	....	17.6	27.1[a]	28.3	89.1	0.032	13.96	8.24	7.02	1.22	14.2[a]
		Green	....	15.7	28.3	29.6	89.8	0.030	13.92	7.49	6.22	1.27	13.1

[a] indicates that the difference is statistically significant.
[b] cc. N/10 NaOH to neutralize 10 cc. juice.

Difference in Physical and Chemical Properties of the Two Sides of Apple

Water Content. In both determinations the water content was slightly but consistently higher on the green than on the blushed side of all varieties tested.

Nitrogen Content. The nitrogen content was consistently higher on the blushed side in the first determination, but there were 2 ties and 1 reverse out of 5 in the second determination. Analysis of the data of both determinations together shows that the nitrogen content of the blushed side was significantly higher than that of the green side. The differences all were very small.

Total Acidity. There was no consistent difference in the total acid content of the two sides of the varieties tested.

Sugar Content. Total and reducing sugars were consistently higher on the blushed side, but there was no consistent difference in the sucrose content of the two sides of the apple fruit.

Tissue Firmness. The blushed side of the fruit was invariably firmer than the green side. The difference was highly significant in all cases.

DISCUSSION

The blushed side of apples is firmer and has higher contents of sugars and total nitrogen and lower water content than the green side; the difference in the acid content of the two sides is inconsistent. The results on the water, sugar, and nitrogen contents are in agreement with those reported by Brooks and Fisher (3) and by Haynes and Archbold (5).

The slower rate of rotting of the blushed side of apples by *Penicillium expansum* and *Sclerotinia fructicola* is correlated with the higher contents of sugars and nitrogen, the lower content of water, and the greater firmness of tissue. If the amount of available food determined the rate of invasion by these two fungi, the blushed side of the apple, containing more sugar and nitrogen, would be expected to rot faster; however, the reverse has been found to be true. It is hardly likely that any of the chemical constituents of the fruit would be so concentrated on the blushed side as to become toxic to the fungi. Nor is it conceivable that a difference of 1 or 2 per cent in the water content of the two sides would cause a change in their susceptibility. Thus is would seem that among the factors tested, a possible explanation for the difference in susceptibility of the two sides of the apple to these two rots lies in their difference in tissue firmness. In the case of *Physalospora malorum* and *Lambertella corni-maris,* however, there was no consistent difference in the rate of rotting of the two sides of the apple fruit. This indicates that the penetration of the fruit tissue by these two fungi was unaffected by the internal factors determined.

In a study of toxic and enzymatic activities of juice expressed from rotted apples, the writer[3] found that the juice of apples rotted by

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[3] Unpublished material.

Penicillium expansum or *Sclerotinia fructicola* contained a considerable amount of protopectinase which macerated thin discs of apple, potato, and carrot while the juice of apples rotted by *Physalospora malorum* or *Lambertella corni-maris* possessed little or no macerating property. On the other hand, juice from apples rotted by *P. malorum* and *L. corni-maris* was found to possess a distinct toxic property indicated by the discoloration of the tissue discs, while the juice from apple rotted by *P. expansum* or *Sclerotinia fructicola* showed no noticeable toxic action. This is in line with the work of Behrens (2), who, in experiments with a *Penicillium* species, shows that the fungus is intercellular and secretes an active middle-lamella-dissolving enzyme, and of Valleau (9), who, in a study of brown rot of plum caused by *S. fructicola,* also demonstrates that the fungus is intercellular and believes that it penetrates through the middle lamella of the suscept cell wall by dissolving the pectic substance with an enzyme.

It has been shown repeatedly that the firmness of fruit tissue is correlated, to a large extent, with the amount of insoluble pectic compounds in the cell wall (Magness and Diehl (6), Appleman and Conrad (1), Plagge *et al.* (7), Haller (4), Smock and Allen (8), and Van Doren (10). Such being the case, the greater tissue firmness of the blushed side would suggest that this side contains more insoluble pectic substance and offers greater resistance to penetration by *Penicillium expansum* and *Sclerotinia fructicola* which are intercellular and make their advance by secreting a middle-lamella-dissolving enzyme, protopectinase. The tissue firmness or the amount of insoluble pectic substance, however, has no effect on the rate of advance of the other fungi, *Physalospora malorum* and *Lambertella corni-maris,* which are presumably intracellular and kill suscept cells ahead of penetration by means of one or more toxic substances.

SUMMARY

It was found that *Penicillium expansum* and *Sclerotinia fructicola* rotted the blushed side of apple fruit more slowly than they did the green side while *Physalospora malorum* and *Lambertella corni-maris* showed no difference in their rate of rotting of the two sides.

The blushed side of apple fruit was found to be firmer and to contain higher contents of sugars and nitrogen and lower content of moisture. The acid content of the two sides did not show any consistent difference.

The slower rate of rotting of the blushed side of apple by *P. expansum* and *S. fructicola* is correlated with higher contents of sugars and nitrogen, lower content of water, and greater firmness of tissue.

As an explanation to account for the difference in the rate of rotting of the two sides of apple by *P. expansum* and *S. fructicola,* it is suggested that the blushed side being firmer possibly contains more insoluble pectic substance and is thus more resistant to the invasion by these organisms which are supposed to be intercellular and penetrate through the cell wall of the suscept by means of enzyme action.

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PHYSIOLOGICAL RESISTANCE TO HALO BLIGHT IN BEANS[1]

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(Accepted for publication June 22, 1941)

During the past 8 years greenhouse and field tests on resistance to halo blight have been carried out with many bean varieties including a number of varieties reported resistant by other investigators. The severity of infection varied with varieties and, although certain varieties tended to escape infection in field tests, it was found that in many cases reportedly resistant varieties proved to be susceptible when inoculated in the greenhouse. However, in the case of several varieties reported resistant by Stapp,[2] together with a few other varieties, the occurrence of small, inconspicuous, brown, circular necrotic spots on inoculated leaves and the absence of typical halo lesions suggested that such varieties may become infected with halo blight without the production of typical halo lesions or serious damage to the plant. Furthermore, no evidence of systemic infection was ever observed in these varieties, although in parallel inoculations other varieties frequently showed veinclearing, stunting, wilting, or other symptoms of systemic infection with halo blight.

These observations strongly suggested the occurrence of physiological resistance to halo blight in certain varieties. Since the definite demonstration of this reaction would be of much value in further understanding the disease and of paramount importance in breeding for resistance to halo blight, experiments were set up to study further the behavior of varieties in which small, necrotic lesions instead of large, chlorotic lesions developed.

Two of these varieties were selected for a more detailed study of their reaction to halo blight infection under various conditions and with different types of inoculation in comparison with 2 susceptible varieties. This paper describes the results of these tests and presents a preliminary report on the behavior of progenies from certain crosses involving these resistant varieties.

MATERIALS AND METHODS

The two varieties selected for the study of their resistance were Red Mexican, a red-seeded, dry-land field bean, and Schwert, Hamburger Markt[3] (Stapp 27, F.P.I. 112,769). The two susceptible varieties used for comparison were Red Kidney and Bountiful.

Bean plants were grown singly in 5-in. clay pots in composted soil in the greenhouse at temperatures varying from 22–24° in the day time and 13–16° C. at night.

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[1] Published with the approval of the Director as Paper No. 293, Journal Series, Nebraska Agricultural Experiment Station.

[2] Stapp, C. Fortgeführte Untersuchungen über die Resistenzverschiedenheiten von Bohnen (*Phaseolus vulgaris*) gegen *Pseudomonas medicaginis* var. *phaseolicola* Burkh. Angew. Bot. 17: 23–42. 1935.

[3] This variety, which will be referred to in this paper as Schwert No. 27, was obtained along with other varieties from Berlin, Germany, in 1935, and the number given each variety is that used by Stapp in his publication, see footnote 2.

The following inoculation methods were used: (a) Leaf and pod inoculations were made by spraying with a bacterial suspension in water. Previous to inoculation, plants were held in an incubation chamber at 24° C. with high humidity for 16 hours. After inoculation the plants were held in the incubator for 6 or 7 hours before removal to a greenhouse bench. In all cases not specified otherwise, plants were leaf-inoculated just as the third trifoliate leaves were unfolding, and pod inoculations were made during pod development. (b) Stem inoculations were made by stabbing twice through a smear of bacteria placed on the bean stems about ¼ in. below the primary leaves. (c) The method used in making germinated seed inoculations was a modification of one described by Stapp[4] and consisted of soaking germinated seeds for 4 hours in a dilute bacterial suspension in water. Seeds were germinated by placing them in a moistened rag-doll germinator held for 60 hours at 27–28°.

Isolates of *Phytomonas medicaginis* var. *phaseolicola* Burkholder, used in these studies, were isolates that, over a period of several years, had continuously produced typical halo-blight symptoms on Red Kidney, Giant Stringless Greenpod, Bountiful, and many other varieties. In all leaf and pod inoculations carried out for the purpose of varietal comparison all 4 varieties in any given test were inoculated with the same bacterial suspension. Forty-eight-hour-old cultures were used in all inoculation tests.

EXPERIMENTAL RESULTS

Four different inoculations were used in the comparative study of the reactions and development of symptoms in the 4 bean varieties: (a) Leaf inoculations, (b) stem inoculations, (c) germinated seed inoculations, and (d) pod inoculations.

Leaf Inoculations. Four or 5 days after inoculation small, water-soaked areas appeared on the under side of leaves of Red Kidney and Bountiful. Within 1 or 2 days typical chlorotic halo areas developed. When heavy inoculations were made and many lesions resulted, part of the inoculated leaf, or in some cases the entire leaf, became chlorotic and wilted. The chlorotic halo lesions usually appeared most prominently from 1 to 2 weeks after inoculation; after this time they gradually became less conspicuous due in part to fading of the green color in the uninfected portion of the leaf.

Following the appearance of primary lesions Red Kidney and Bountiful plants often showed symptoms of systemic infection. These symptoms were first manifested by a slight yellowing and vein-clearing of one or more young leaflets near the growing point. Vein-clearing symptoms ordinarily appeared 8 to 12 days after inoculation and were, in turn, frequently accompanied or followed by stunting, wilting, and premature death of the plants. The development of the symptoms of systemic infection depended to a large extent upon the age and the general vigor of the plant at the time of infection. Inoculation of young, vigorously growing plants gave the highest proportion of systemic infection.

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[4] Stapp, C. Verfahren zur Prüfung von Bohnen (*Phaseolus vulgaris*) auf Resistenz gegen *Pseudomonas medicaginis* var. *phaseolicola* Burkh., dem Erreger der Fettfleckenkrankheit. Angew. Botanik, 15: 241–252. 1933.

On the varieties Red Mexican and Schwert No. 27 the symptoms developed as small, circular, brownish, inconspicuous necrotic lesions that never enlarged to any noticeable extent. Inoculated leaves did not become yellow or wilted, even though in some cases the number of necrotic lesions was large. No symptoms of systemic infection were ever noted on plants of these 2 varieties, even though infected plants were held until mature. Figure 1 presents a comparison of the leaf symptoms on Schwert No. 27 and the susceptible variety Red Kidney.

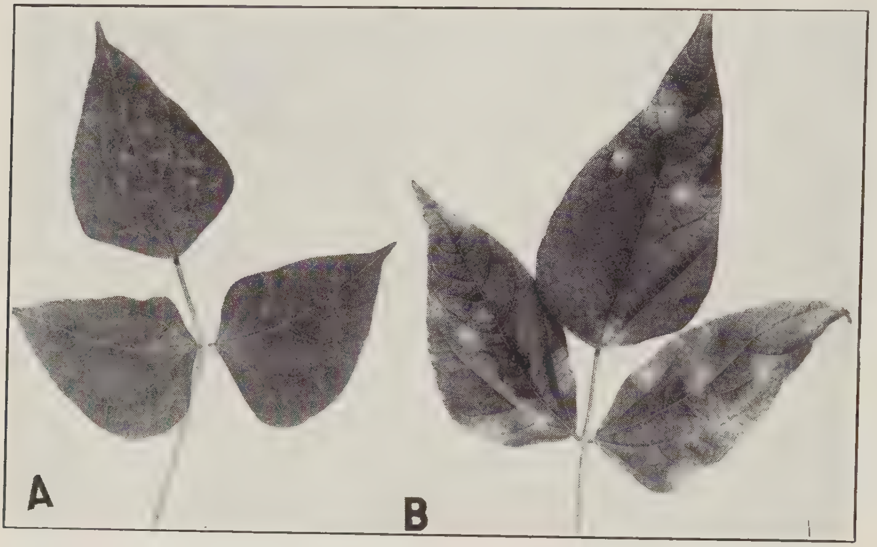

FIG. 1. Symptoms resulting from leaf inoculation with the halo-blight organism. Small, necrotic lesions occur on the leaves of the resistant variety Schwert No. 27 A in contrast with the large halo lesions on the leaves of the susceptible variety Red Kidney B.

In the course of experiments with these 4 varieties, inoculation tests were carried out with plants of all ages from early seedling stage to those nearing maturity. No indications were obtained that the reactions were influenced by age of plant, excepting, of course, that when nearly mature plants were inoculated no leaf symptoms developed.

In a recent paper Goss[5] reported that, under high temperature conditions (28–32° C.), lesions that lack halos appear on leaf-inoculated plants of Red Kidney and U.S. Refugee No. 5, although at lower temperatures (16–20° C.) typical halos were formed on these varieties. In an attempt to determine whether the development of necrotic halo-less lesions on Red Mexican or Schwert No. 27 were influenced by temperature relations, the following experiment was carried out: Forty young plants of each of the 4 varieties were leaf-inoculated. Six hours after inoculation 10 plants of each variety were placed in the greenhouse in temperature cases held at 16, 22, and 28 degrees C. Within 7 days after inoculation typical halo lesions appeared on leaves of Red Kidney and Bountiful held at usual greenhouse temperatures and in the 22° case, whereas small, necrotic, halo-less lesions developed on Schwert No. 27 and Mexican Red.

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[5] Goss, R. W. The relation of temperature to common and halo blight of beans. Phytopath. 30: 258–264. 1940.

At 28° C. lesions without halos appeared 7 days after inoculation on Red Kidney and Bountiful, as reported by Goss for the varieties Red Kidney and U.S. Refugee No. 5. On leaves of Red Mexican and Schwert No. 27 haloless necrotic lesions appeared about 10 days after inoculation at this temperature.

Since Goss reported that the most prominent halo lesions appeared on susceptible plants held at 12–20° C., it, therefore, seemed probable that if lesions with halos ever occurred on leaves of Red Mexican or Schwert No. 27 they would appear in this range of temperatures. In tests on the effect of temperature only small, necrotic halo-less lesions developed on leaves of Red Mexican or Schwert No. 27 at 16° C. 12 days after inoculation, although large, prominent halo lesions appeared on leaves of Red Kidney and Bountiful plants inoculated at the same time and held in the same temperature-controlled cases. Furthermore, no halo lesions developed on leaves of Red Mexican or Schwert No. 27 although they were held at 16° C. for a period of more than a month after inoculation.

These tests showed that halo symptoms do not appear on inoculated Red Mexican or Schwert No. 27 plants at 28, 22, or 16 degrees C., although halo lesions do occur on Red Kidney and Bountiful at 22 and 16 but not at 28° C. It was concluded from these experiments that necrotic, halo-less lesions developed on leaves of Red Mexican and Schwert No. 27 over a wide range of temperature, and that the reaction was different from the one reported by Goss.

Stem Inoculations. Ten equally old plants of each variety were selected and stem-inoculated when the first trifoliate leaves were about half-expanded. Additional plants to serve as controls were punctured with sterile needles and still other plants were not treated.

Four days after inoculation by the stem-puncture method Red Kidney plants started wilting and began to lose their dark green color. Bountiful plants showed similar symptoms a day or two later. Plants of these 2 varieties that had been punctured with sterile needles remained healthy, as did the untreated controls. Inoculated Red Kidney and Bountiful plants showed vein-clearing, yellowing, wilting, and severe stem cankers with abundant bacterial exudate, and within 3 weeks all were dead.

The behavior of stem-inoculated plants of Red Mexican and Schwert No. 27 was in marked contrast to the above. Although small, watersoaked areas and internal necrosis developed for a short distance around the point of inoculation, the plants continued to grow without the development of any additional symptoms. The punctured controls and untreated plants remained healthy. Figure 2 presents a photograph of plants of the 4 varieties 15 days after they were stem-inoculated.

It was concluded from these tests that systemic infection with halo blight is not induced in Red Mexican or Schwert No. 27 by stem inoculation with strains of the halo-blight bacteria that produced systemic infection in Bountiful and Red Kidney plants. Since these halo strains were the most pathogenic in the collection, it seems highly probable that systemic infection cannot be induced in the two varieties.

Germinated-seed Inoculation. Twenty germinated seeds of each of the 4 varieties were immersed for 2 hours in a bacterial suspension of the halo-blight organism in water. Additional germinated seeds, to serve as controls, were immersed for the same length of time in tap water. After immersion all seeds were planted in soil in clay pots. Within several days water-soaked lesions could be seen on the cotyledons as they unfolded aboveground on all 4 varieties grown from inoculated seed. Later on, lesions appeared on the primary leaves of many plants in all 4 varieties. Within a week nearly all plants of Red Kidney and Bountiful showed symptoms of systemic infection, and, 3 weeks after inoculation, all plants of these 2 varieties were dead. Inoculated Schwert No. 27 and Red Mexican plants, on the other hand, showed no symptoms of systemic infection, but grew to maturity and produced a crop of apparently healthy pods.

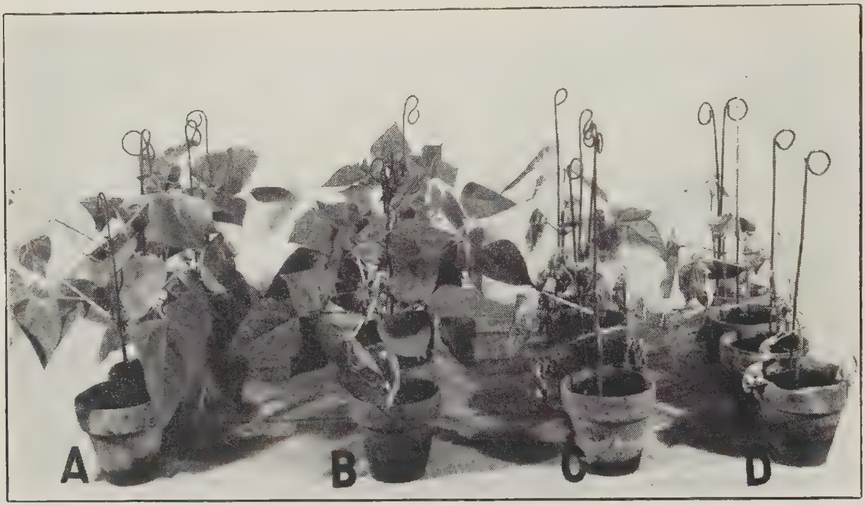

FIG. 2. Bean plants of the four varieties 15 days after stem inoculation with the halo-blight organism. A. Schwert No. 27. B. Red Mexican. C. Red Kidney. D. Bountiful. The two resistant varieties showed no symptoms of systemic infection and produced a normal crop of beans.

These tests further demonstrate a physiological resistance in Red Mexican and Schwert No. 27, as shown by the failure of these varieties to develop systemic infection, even though inoculated and infected in the early seedling stage.

Pod Inoculations. The striking differences that had been obtained in leaf symptoms and in the experiments dealing with systemic infection suggested that some differences might be found in pod lesions on the varieties being studied. Accordingly, 20 plants of each of the 4 varieties were grown in 7-inch clay pots, one plant to each pot, to insure strong, vigorous growth and abundant pod production. When some of the earliest pods of each variety were nearly full-grown, and at a time when each variety was still producing new pods, they were inoculated by the same methods used for leaf inoculations. Within a week after inoculation typical water-soaked lesions appeared on the pods of Red Kidney and Bountiful plants. These enlarged and became typical halo-blight pod lesions. Pods on Schwert No. 27 and Red Mexican, however, developed small, discrete, dry, necrotic spots, which did not enlarge to any extent and, after several weeks, looked like rusty-colored spots caused by mechanical injury. Figure 3 presents photographs of typical pods of the 4 varieties inoculated with halo blight.

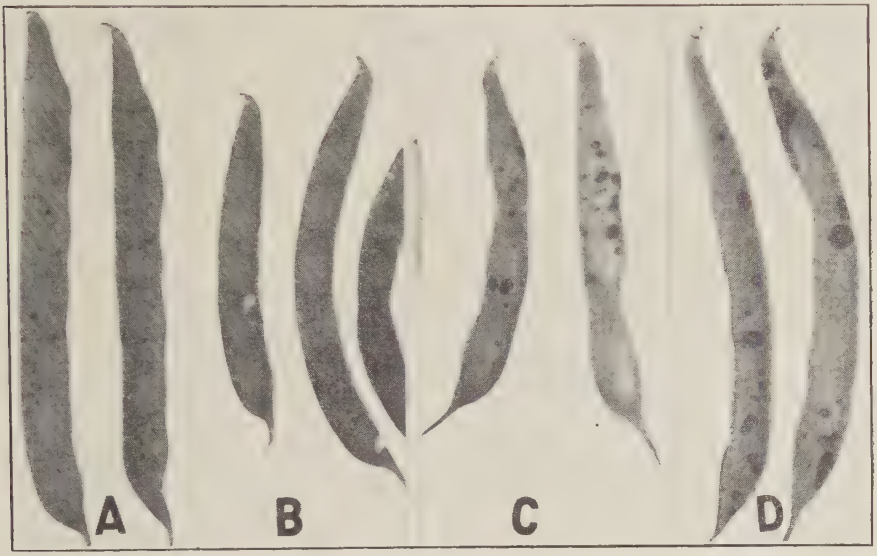

FIG. 3. Symptoms resulting from pod inoculations of the four varieties. A. Schwert No. 27. B. Red Mexican. C. Red Kidney. D. Bountiful. The small, discrete, necrotic lesions on the 2 resistant varieties contrast with the larger, water-soaked, coalescing lesions containing bacterial exudate on the pods of the 2 susceptible varieties.

It was concluded from this test that pod lesions on Red Mexican and Schwert No. 27 were markedly different from those produced on susceptible varieties, such as Red Kidney or Bountiful, and that the lack of water-soaking and the small size of the lesions further confirmed the conclusion that these varieties possessed physiological resistance to halo blight.

OTHER RESISTANT VARIETIES[6]

A large number of varieties have been tested either in the field or greenhouse, and, while the detailed results cannot be presented in this paper, it should be noted that the following varieties have shown resistance when inoculated by one or more of the methods previously described.

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[6] The variety Princess of Artois was obtained from Canada through the courtesy of T. F. Ritchie. Startler and White Seeded Runner were obtained from Western Australia through the courtesy of W. P. Cass Smith. Drouth Resistant was received from the McFayden Seed Co. of Canada.

Schwert Nordstern (Stapp 82, F.P.I. 112,771) reacted in a manner similar to the 2 varieties, described in the foregoing as resistant, when inoculated by all 4 methods.

Great Northern, Princess of Artois, Robust, Kaiser Wilhelm (Stapp 100, F.P.I. 112,773) and Marktsieger (Stapp 38, F.P.I. 112,700) were all resistant to germinated-seed and stem inoculations, but gave variable symptoms with leaf and pod inoculations. White Seeded Runner and Zucker-Brech (Stapp 196, F.P.I. 112,775) were resistant to leaf, pod, and stem inoculations, but developed some systemic infection and halo symptoms when germinated seed were inoculated. Startler was resistant with seed, leaf, and stem inoculations, but variable symptoms occurred on the pods; while Blue Lake was resistant to stem and pod inoculations, but gave variable symptoms on the leaves.

In other tests in which leaf and pod inoculations only were used, California Pink and Blue Pod showed resistance and in similar tests no infection was obtained on Drouth Resistant or Arikara Yellow.

BREEDING TRIALS

It seemed possible that crosses between either of the resistant varieties Schwert No. 27 or Red Mexican and desirable commercial varieties, such as Asgrow Stringless Green Pod or Landreth Stringless, would show promise of combining resistance to halo blight with desirable horticultural characters. Accordingly, a number of crosses were made and while sufficient data are not at hand for establishing definite genetical relationships, the preliminary information on such crosses is of interest.

In crosses of either Schwert No. 27 or Red Mexican with Asgrow Stringless Green Pod or Landreth Stringless, all F_1 plants, when inoculated, produced both typical halo lesions and necrotic non-halo lesions. In other crosses with the same parentage, however, segregation occurred in subsequent generations up to the F_5 so that the progeny of certain selections showed necrotic lesions almost exclusively, while those of other selections from the same crosses showed typical halo lesions.

It is, therefore, concluded probable that resistance to halo blight can be combined with desirable horticultural characters, although back-crossing probably will be necessary before desirable resistant varieties of commercial value are produced. Work is being continued along this line.

DISCUSSION

Many bean varieties, reportedly resistant to halo blight in the field, have become severely infected when inoculated in the greenhouse under controlled conditions. Certain other varieties have shown what appears to be a true physiological resistance when subjected to similar tests. Undoubtedly there are several genetic factors involved in the host-parasite relationship with halo blight. It is likely that some factors exercise control over the entrance of the organism, while others influence the action of toxic substances and the movement and multiplication of the bacteria in the host.

The utilization of the type of resistance here reported in the development of new bean varieties should greatly aid in the control of this disease. Relatively little damage occurs to the yield or the quality of the marketable crop from such infections as have been described. In addition, the small size of the lesions would tend to reduce the production of bacterial exudate and thus limit the amount of inoculum and resultant spread of the disease.

SUMMARY

The development of small, inconspicuous, necrotic lesions on leaves of some bean varieties inoculated with halo blight, instead of the large chlorotic spots that develop on susceptible varieties, suggested that these varieties possess physiological resistance to the disease. These symptoms occurred on leaves of Red Mexican and Schwert No. 27 over a wide range of temperatures, 16, 22, and 28 degrees C., and on plants of all ages from early seedling to plants nearly mature. The inoculation of germinated seed by soaking in a bacterial suspension or of young plants by stem punctures failed to bring about systemic infection. Pod inoculation produced small, rusty-colored, necrotic lesions on these 2 varieties instead of the usual large, water-soaked lesions. It is concluded that these varieties, and others reacting similarly, possess true physiological resistance to halo blight.

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THE EFFICACY OF CERTAIN NEMATOCIDES IN THE CONTROL OF ONION BLOAT IN MUCK SOIL[1]

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(Accepted for publication June 27, 1941)

In a former paper[2] the writers discussed the recent introduction into New York State of the disease of onions (*Allium cepa* L.) known as "bloat," caused by the bulb or stem nematode *Ditylenchus dipsaci* (Kühn) Filipjev. Attempts to eradicate it from the onion fields were described; where steam sterilization was no longer practicable, sulphur and chloropicrin were said to have given some promise as soil nematocides in preliminary field trials on a few farms. Since then, in the early summer of 1940, there has been an increase in the number of onion fields infested with *D. dipsaci*, due apparently to excessive precipitation. The problem has, therefore, become more acute, particularly where growers do not practise a rotation.

It seemed feasible to test the efficacy of certain chemicals as nematocides in onion fields, on the basis of their success in other crops and with other species of nematodes. The use of chloropicrin, ethylene chloride, and mixtures of the two had been reported by Chitwood,[3, 4] as applied to sandy-loam soil infested with the daffodil strain of *Ditylenchus dipsaci* and fields infested with the root-knot nematode *Heterodera marioni* (Cornu) Goodey. The results of the first of a series of experiments testing these and other chemicals for the control of onion bloat are given herewith.

PLAN OF EXPERIMENT

One hundred metal cylinders 17 in. long and 2 ft. in diameter, open at both ends, were driven into muck soil to a depth of 12 in. in a field near Watkins, New York. There were 20 experimental treatments (including 3 types of controls) with 5 replications of each, all assigned at random among the 100 cylinders or plots. Three types of inoculum were used and the experiment is outlined, according to inoculum, in the following paragraphs.

(1) In 5 treatments diseased onion bulbs were buried in the soil and served as the source of inoculum. One of these treatments was given no chemical and served as a control, while the 4 remaining treatments received the following chemicals:[5] Chloropicrin at 2cc. per injection to a depth of

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[1] We are indebted to Mr. L. B. Reed of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, for aid in the statistical analysis.

[2] Newhall, A. G., and B. G. Chitwood. Onion eelworm rot or bloat caused by the stem or bulb nematode, *Ditylenchus dipsaci.* Phytopath. **30**: 390–400. 1940.

[3] Chitwood, B. G. Ethylene chloride for sterilization of soil against nematodes. Florists' Exchange **95**: 9. 1940.

[4] Chitwood, B. G. Soil treatments with volatile liquids for the control of nematodes. Phytopath. **31**: 818–824. 1941.

[5] For method of making treatments see:

Chitwood, B. G. A rapid method for determining *K* values of nematocides. Proc. Helminthol. Soc. Washington **6**: 66–70. 1939; and Frames for spacing injections of soil nematocides. *Ibid.* **6**: 70–73. 1939; and

Taylor, A. L. Efficient spacing of soil fumigants for field applications. *Ibid.* **6**: 62–66. 1939.

6 in., spaced 9 × 10½ in.; chloropicrin-ethylene chloride (1:9) at 10 cc. per injection to a depth of 6 in., spaced 9 × 10½ in.; sulphur at 1¼ tons per acre mixed with top 6 in. of soil; and sulphur at 2½ tons per acre.

(2) In 14 treatments screened infested soil alone served as inoculum. One of these treatments served as a control; 4 treatments were identical with the chemical treatments given in paragraph (1) and the remaining 9 chemical treatments are listed in table 1.

(3) In one treatment diseased onion tops (leaves) served as the source of inoculum. No chemical was applied to this treatment.

TABLE 1.—*Results of various soil treatments on the production of onions and the control of Ditylenchus dipsaci in muck soil (average of 5 replications)*

Treatment	Mean weight of 30 bulbs		Infected bulbs (with bulbs as inoculum)[a]	Efficacy of treatment
	with soil as inoculum	with bulbs as inoculum		
	Grams	*Grams*	*Mean number*	*Per cent*
Control	2909	1298	6.6	0
Chloropicrin (2 cc. 9 × 10.5″)	1324[b]	1856	0.2[c]	97[d]
Chloropicrin-ethylene chloride 1:9 (10 cc. 9 × 10.5″)	1994[e]	1870	0.0[f]	100[g]
Sulphur 1¼ tons per acre	2128	1260	4.8	38
Sulphur 2¼ tons per acre	966[b]	708	4.2	31
Chloropicrin (1 cc. 9 × 10.5″)	2864	....	......	....
Chloropicrin (3 cc. 9 × 10.5″)	692[b]	....	......	....
Chloropicrin-ethylene chloride 1:4 (5 cc. 9 × 10.5″)	2986	....	......	....
Ethylene chloride (10 cc. 9 × 10.5″)	2904	....	......	....
Crotonaldehyde (10 cc. 9 × 10.5″)	3230	....	......	....
Crotonaldehyde (10 cc. 6 × 7″)	3018	....	......	....
Mesityl oxide (10 cc. 9 × 10.5″)	2814	....	......	....
Mesityl oxide (10 cc. 6 × 7″)	3016	....	......	....
Carbon disulphide (10 cc. 9 × 10.5″)	2892	....	......	....
Controls (onion tops added)	2528	....	0.2	....

[a] No infected bulbs in any case where soil alone was used as inoculum.

[b] Weight reduced odds of 999: 1.

[c] Disease reduced odds of 19: 1.

[d] Efficacy as based on binomial distribution formulae not less than 86 per cent with odds of 19: 1.

[e] Weight reduced, odds of 19: 1.

[f] Disease reduced, odds 99: 1.

[g] Efficacy as based on binomial distribution formulae not less than 91 per cent with odds of 19: 1.

At the time the treatments were made on October 5, 1939, the moisture in several samples of the soil ranged from 38 to 52 per cent of the dry weight, the soil temperature varied from 16 to 19° C., while the soil reaction was pH 4.6 to 5.8. Enough water was added to each plot immediately after treatment to facilitate the retention of volatile materials. On April 27 of the following year 5–10-10 fertilizer was applied at the rate of 1200 pounds per acre and 30 healthy onion sets of variety Ebenezer were planted in each plot. Harvest records on mature onions were made on August 6.

EXPERIMENTAL RESULTS

(1) *Control of Ditylenchus dipsaci.* In none of the treated plots or controls where infested but sifted muck was employed could a single onion infected with *D. dipsaci* be found. One infected onion was found in one plot of the control series in which infected onion tops had been buried. The standard error of the difference for comparison of 5 paired treatments (including controls) with or without buried diseased onions equals 0.5161; the minimum mean difference with odds of 999 to 1 is 1.771. The mean number of diseased bulbs per plot where diseased onions were buried was 3.16, and, since none was found in corresponding treatments lacking buried diseased onions, this figure is significant with considerably greater odds than 999 to 1.

Analysis of variance of onion bulbs infected with *Ditylenchus dipsaci* after soil treatment, with onion bulbs as source of inoculum, gave a standard error of the difference for comparison of treatment means of 2.305. The mean difference required for significance with odds of 19:1 equals 4.8 and with odds of 99:1 equals 6.6. Means of treatments on soil containing buried infected onion bulbs are given in table 1. Two treatments are significant, the 2 cc. chloropicrin and the 10 cc. mixture of chloropicrin-ethylene chloride (1:9).

(2) *Other Diseases.* Small percentages of miscellaneous troubles, including fusarium bulb rot, bacterial rot, and onion maggot, were found throughout the plots. Analysis showed that these were not significantly affected by any of the treatments. However, where *Ditylenchus dipsaci* was present in a plot the number of rotten onions was increased. This was apparently due to the entrance of *Ditylenchus dipsaci*-infected bulbs by secondary invaders. It appears probable that this miscellaneous residuum of bulbs infected with some type of disease amounting to means of 0.0 to 2.0 per plot was introduced with the sets.

(3) *Effect on Yield of Bulbs.* The total weight per plot was taken and an analysis of variance conducted. The standard error of the difference for comparison of treatments equals 425.252 grams. The mean differences required for significance at various levels are as follows: with odds of 19:1, 848 grams, with odds of 99:1, 1125 grams, with odds of 999:1, 1459 grams. Comparing the various treatments in which onions served as a source of inoculum, one notes rather larger differences in some instances but these differences are dependent on both treatment and degree of infestation. Comparing the various treatments in which soil served as a source of inoculum, 4 treatments differ significantly from the corresponding control. These treatments are chloropicrin at a dosage rate of 2 and 3 cc. per injection, sulphur at a rate of 2½ tons per acre and chloropicrin-ethylene chloride at a dosage rate of 10 cc. per injection. All of these treatments were detrimental in the present experiment, the chloropicrin-ethylene chloride mixture being the least injurious.

DISCUSSION

Sifted muck soil from a field showing heavy infestation with *Ditylenchus dipsaci* in 1939 did not prove effective as a source of infection in the experiment. It was necessary to add further inoculum in the form of diseased onions or, in one case, leaves from diseased onions. The writers (*loc. cit.*) have reported the failure of previous attempts to obtain infection on seedlings grown in this same sifted soil. The latter half of the summer of 1939 was unusually dry, resulting in warm soil temperatures which may have contributed to the apparent absence of nematodes free in the soil.

Sulphur mixed with the soil in the fall, even at 2½ tons per acre, did not result in a significant reduction of bloat in the next season's crop when diseased onions had been previously buried in the soil.

Chloropicrin, at a dosage rate of 2 cc. in holes 9 × 10½ in. apart ($390 per acre) reduced bloat significantly. The efficacy was 97 per cent.

Chloropicrin-ethylene chloride mixture (1:9), at a dosage rate of 10 cc. ($294 per acre), eradicated the disease completely.

Sulphur, at 2½ tons per acre and chloropicrin at a dosage rate of 2 and 3 cc. per injection (9 × 10½), caused marked reduction in crop weight. Sulphur, at the rate of 1¼ tons per acre, caused some reduction in crop weight (not significant in this experiment). In experiments with 1¼ tons of sulphur per acre, reported elsewhere,[7] the reduction in crop weight was significant, even in the second year. Chloropicrin-ethylene chloride (1:9), at 10 cc., caused some reduction in crop yield, but ethylene chloride at 10 cc. caused no reduction in yield.

CONCLUSIONS

After a dry season infected onions in the soil are the chief source of inoculum of *Ditylenchus dipsaci.* Infected onion tops also may harbor this nematode to a slight extent.

Sulphur, applied to the soil in the fall, was found to be of no significant value as a nematocide in the present experiment. Heavy applications greatly reduced crop weights.

Fall applications of chloropicrin at the rate of 2 cc. in holes 9 × 10½ in. apart and a mixture of chloropicrin and ethylene chloride (1:9) at the rate of 10 cc. in holes 9 × 10½ in. apart both gave very highly significant reduction of onion bloat. The latter treatment was better than the former one but the difference was not significant.

Since ethylene chloride alone was used only with sifted soil as inoculum, and since this inoculum was shown to be inadequate, the efficacy of this material by itself in muck soil remains to be proved. It seems highly probable that when used at a rate of 10 cc. in holes 9 × 10½ in. apart it would give adequate control of *Ditylenchus dipsaci,* as judged by the senior writer's recent results[8] concerning its application in soil infested with the narcissus strain of *D. dipsaci.* Ethylene chloride at this rate would cost only $110 per acre. It should be given further trial.

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[7] Newhall, A. G., and B. G. Chitwood. The Status of Onion Bloat in 1940. U.S.D.A. Bureau of Plant Industry Plant Dis. Rptr. 24: 350–351. 1940.

[8] See footnote 4.

Chloropicrin, either by itself or in mixture, sometimes reduced onion crop weights. It seems possible that it may have been adsorbed on the carboniferous muck soil. Perhaps the metal containers contributed to its retention. Field application in a similar manner produced no crop reduction.

No evidence of crop stimulation was noticeable from any treatment and the only adverse effects on growth were in the sulphur and chloropicrin-treated plots. Field applications of sulphur have also given consistent reductions in crop weights. Some of these have been reported as persisting into the second season after application.[9]

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[9] See footnote 7.

ASCOCHYTA MAJALIS IDENTIFIED ON LILY OF THE VALLEY IN THE UNITED STATES[1]

ANNA E. JENKINS

(Accepted for publication June 27, 1941)

The first known record of *Ascochyta majalis*, causing a leaf spot of lily of the valley, in the United States was obtained in August, 1940, when a grower in Bucks County, Pennsylvania, sent specimens to the United States Department of Agriculture. He sought advice concerning this diseased condition of his planting. He stated that the bed, about 50 × 20 ft. in size, had been maintained for at least 15 years with no trouble previously from plant disease. At this time, however, near the center of the plot an elongated area about 20 × 2 ft. became entirely discolored, and the green leaves outside this area were more or less generally spotted.

The specimens received in Washington showed striking leaf spots scattered over the blade. They were often elliptical or elongated, reaching 2 cm. in the longer diameter. The spots were brown,[2] sometimes bordered by purple, and surrounding the border was generally a more or less indefinite zone of bright-green (Fig. 1, A and B). Scattered over the surface of the lesions were minute elevations, which proved to be pycnidia (Fig. 1, C). These occupied nearly the entire thickness of the leaf and their color made them visible through the leaf tissue. They were spherical to flattened, bearing 2-celled or occasionally 3- or 4-celled conidia (Fig. 1, D).

The fungus, recognized as a species of *Ascochyta*, proved to be in agreement with *A. majalis*. This is one of the fungi discovered by Massalongo (3) in Verona, Italy, and the description reads as follows:

"*Ascochyta Majalis* sp. nov.—Maculis fulvo-rubiginosis oblongis ambitu lutescentibus; perithecis membranaceis hypodermis amphigenus osculo minute pertusis 140 : 180 μ. in diametro; sporalis hyalinus saepe quadriguttulatis subcylindraceis, raro curvulis utrinque rotundatis, 1-septatis, ad septum interdum subconstrictis 18 : 24 × 4 : 6 μ.

"In foliis subemortuis *Convallariae Majalis*, in cultis vici 'Tregnago': Oct. 1899."

Massalongo again collected this fungus at Tregnago in September, 1901, and this collection was issued in D. Saccardo's Mycotheca Italica as Number 960.

The only other available record of the fungus in Europe is represented by its discovery in the Austrian Alps in July, 1907, by Otto Jaap (4). His record of the fungus, apparently on wild lily of the valley, reads as follows:

"*A. majalis* Mass. Auf lebenden Blättern von *Convallaria majalis* auf der Mendel am Wege zum Penegal.—Blattflecken weichen etwas ab von der Beschreibung; stimmt sonst gut."

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[1] Paper presented at the thirty-second annual meeting of The American Phytopathological Society, Philadelphia, Pennsylvania, December 27 to 31, 1940 (2).

[2] On the dry specimen "Hessian brown" according to Ridgway (7).

An actual comparison of the specimen from Pennsylvania with that from Italy collected in 1901 shows that the fungus from the United States is entirely in agreement with that from Europe. In either case the spores tend to be yellowish rather than entirely hyaline. Owing to their sticky walls they may form agglutinated masses and may not be easily separated from one another. The spores shown in figure 1, E, are from the specimen of 1901 from Italy. It will be noted that a 3-celled spore is represented.

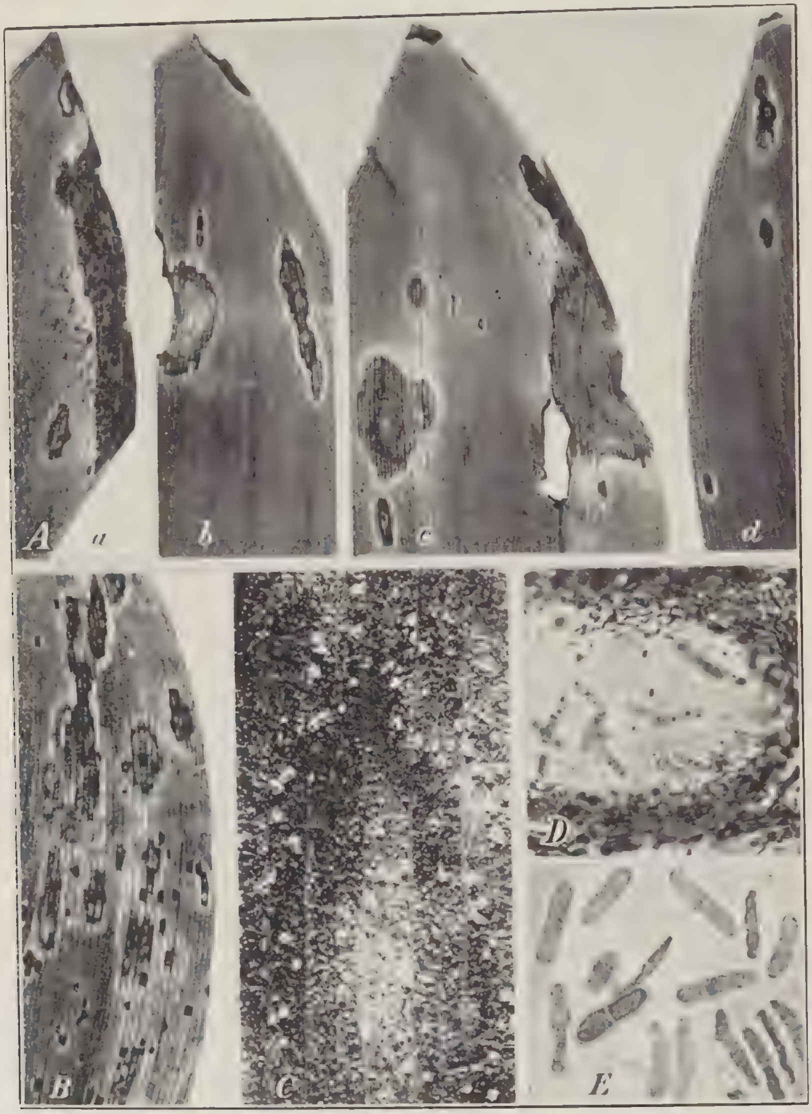

FIG. 1. A, *a–d*. *Ascochyta majalis* on lily of the valley from Bucks Co., Pa., September, 1940. × 1. B. Part of the leaf from the type locality (Italy). × 1. C. Central part of the large leaf spot in A, *c*, showing pycnidia of the *Ascochyta*. × 5. D. Part of a pycnidium, with spores, from the specimen from Pennsylvania. × 300. *E*. Spores from the specimen from the type locality, Verona, Italy, September, 1901 (D. Sacc. Fungi Italica No. 960). × 500. Photographs by M. L. F. Foubert.

On the material from Pennsylvania the *Ascochyta* was clearly in prominence and there seemed no question that it was responsible for the severely diseased condition in the bed of the lily of the valley concerned. The owner stated that certain younger and more recent beds were apparently healthy; moreover, that in no case had plants from outside sources been introduced into his highly prized and theretofore successful plantings.

The manner in which this little-known fungus reached the bed of lily of the valley is, of course, unknown. But, in its origin in the United States, it would appear that it is an introduction from Europe.

Isolations of the *Ascochyta* from Pennsylvania were made by means of tissue cultures. The plantings were first dipped in a solution of mercuric chloride (1:1000) and then rinsed in sterile water. Potato-dextrose agar medium was employed, and on this substrate a dull white cottony fungus growth soon developed. Later, yellowish, sticky masses of conidia of the *Ascochyta* were present, although pycnidia were not distinguished.

In one case an organism other than the *Ascochyta* was obtained in culture. The planting, which was part of a discolored area on a leaf base, gave no growth for several days, when a pure culture, supposedly of *Kabatiella microsticta* Bubak (1), began to develop. This fungus, already known to the writer and previously reported (5) from the United States, was also formerly discovered in Europe. The type collection, from Bohemia, 1905, by Kabát, is represented by Kabát and Bubák, Fungi Imperfecti Exsiccati No. 435, of which a specimen is available. From Pape's (6) account of a disease outbreak in cultivated lily of the valley in Germany, it seems to the present writer that the *Gleosporium* sp. to which the disease is attributed may prove to be *K. microsticta*.

In autumn, 1940, only this fungus and no *Ascochyta* was found on leaf spots on freshly collected specimens of lily of the valley from two widely separated gardens in Washington, D. C.

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6. PAPE, H. Gloeosporium-krankheit in Maiblumenkulturen. Gartenw. 31: 344–346. 1927.
7. RIDGWAY, R. Color standards and color nomenclature. 42 p., 52 colored plates. (Washington) 1912.

BACTERIAL WILT OF DENT CORN INBREDS

Charlotte Elliott[1]

(Accepted for publication July 14, 1941)

Losses from bacterial wilt of dent corn have, until recently, been considered of minor importance. Increasing evidence, however, of the importance of this disease on dent corn developed during the wilt epidemics of sweet corn in 1932 and 1933 and later in 1937 and 1938. During these epidemics, bacterial wilt assumed an unusual, late-season importance on dent corn in the form of a leaf blight. It was reported that late leaf blight in many fields reduced the green leaf area as much as 25 per cent,[2] and that in such fields grain yields were greatly reduced.

On both dent and sweet corn, bacterial wilt begins from the feeding injuries, on the leaves, produced by the corn flea beetle (*Chaetocnema pulicaria*). Although the manner of infection and general symptoms on both kinds of corn are the same, the time and type of injury are different. On sweet corn, early systemic infection is the important phase of the disease. On dent corn, local leaf-blight lesions on maturing plants do the greatest damage.

The young plants of some dent-corn lines show pronounced feeding injury; but, in spite of such injuries, few wilt lesions develop. At this stage in their development the young dent-corn plants appear to be much more resistant to infection than young sweet-corn plants. Records taken at Arlington Experiment Farm, Arlington, Virginia, about the middle of June, have shown that, in 1939, of 15,000 dent-corn inbred plants, 1.5 per cent were infected, as compared with 27 per cent infected sweet-corn plants; and that at the comparable time in 1940, of 12,000 dent-corn plants, 0.7 per cent were infected, as compared with 35 to 46 per cent infected sweet-corn plants. In both seasons the young plants of both dent and sweet corn were in adjacent plots in the same field. On the whole, therefore, young dent-corn plants appear to be much more resistant to natural infection than those of sweet corn.

A change in the resistance of dent-corn plants to bacterial wilt apparently takes place in late July and August, after the plants have tasselled. At this season, in Virginia and Maryland, as many as 80 per cent or more of the corn flea beetles are carrying the wilt organism and can transmit the bacteria to the dent-corn leaves. Local, light-green to yellow spots and streaks begin to appear on the lower leaves. Through August and September, these leaf spots may increase rapidly in size and number on susceptible lines, and spread from the lower to the upper leaves. Infected leaf tissue soon dies and becomes dry. Heavily infected leaves, whipped by the wind, become torn and ragged. In heavily infected varieties, the light-colored dead tissue gives a row or field the appearance of having been frosted. Very susceptible varieties may become so heavily infected that whole plants become prematurely dry.

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[1] Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Koehler, Benjamin. Several corn diseases unusually prevalent in Illinois. Pl. Dis. Rptr. 22(18): 374. 1938.

It is this late leaf blight in July, August, and September that is the important phase of bacterial wilt on dent corn. In susceptible varieties, losses of green leaf area have been estimated as large as 50 per cent, and such infection apparently increases susceptibility to diplodia stalk rot.[3]

With the increasing acreage of hybrid corn, it is important to know the reaction to bacterial wilt of inbred parent lines, as well as of the hybrids. Preliminary tests of the reaction of inbred lines of dent corn to bacterial wilt were begun at Arlington Experiment Farm in 1939 and continued in 1940. In order to test their relative resistance in early, as well as late, stages of growth, records were kept of the amount of infection resulting from field inoculations of young plants and of the amount of leaf blight late in the season on the same maturing inbreds. Ten plants each of 800 to 900 inbred lines were inoculated when 10 to 12 inches tall to determine differences in susceptibility in early stages of growth. The results were recorded in ratings of 1 to 5, 1 being resistant; 2, moderately resistant; 3, moderately susceptible; 4, susceptible, and 5, very susceptible. Later in the season leaf blight developed naturally on noninoculated plants in the same rows, and differences were recorded by the same ratings, 1 to 5. The results of these ratings are given in table 1.

Inoculations of the young plants were made by hypodermic injections into the stalk about 1 inch above the ground. The inoculum was a water suspension of 3 virulent cultures of *Xanthomonas stewarti* (E. F. Smith) Dowson. In the 1939 inoculations the fibrovascular bundles were wounded somewhat more than in 1940. The results of inoculations for 1939 and 1940 are given in the 2 left-hand columns and the leaf-blight ratings in the right-hand columns. The inbreds are in the order of their relative resistance to the artificial inoculation of the young plants. While the results are not identical for the 2 years they appear to be reasonably uniform.

As shown in table 1, the inbreds vary in susceptibility to inoculation in the seedling stage from resistant (1) to very susceptible (5). At least 12 lines were very resistant in both seasons. Eight were moderately susceptible (3) and KYS was very susceptible (5). The plants of KYS, when inoculated, made no further growth and wilted and died.

The results of the inoculations show greater variability in 1940 and, in most instances, less infection than in 1939. This lighter infection is probably due to the less vigorous inoculation methods used in 1940.

From the leaf-blight records in the right-hand columns, with one exception, these inbreds are all more susceptible to leaf blight as the plants are maturing than to inoculation in early stages of growth. Inbred Ia. L317

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[3] Holbert, J. R., Charlotte Elliott, and Benjamin Koehler. Bacterial leaf blight of dent corn. Phytopath. 23: 15–16. 1933.

TABLE 1.—*Bacterial wilt ratings of a selected group of inbred lines of dent corn grown at Arlington Experiment Farm, Arlington, Virginia, 1939 and 1940*

Inbred lines	Wilt ratings[a] on			
	Young plants hypodermically inoculated		Mature plants naturally infected with leaf blight	
	1939	1940	1939	1940
Ky. 27	1	1	2	1–2
US 23	–	1	2	2
Mo. G	1	1	2	2
Ky. 39	–	1	2	2
US 24	–	1	3	2
US 21	1	1	3	2
Ill. Hy	1	1	3	2
Ind. 38–11	1	–	3	3
Ind. P8	1	1	3	3
US 41	1	1	3	3
K58	1	–	4	2
Ky. 30A	1	1	4	2
Ia. I205	1	1	4	2
Ohio 51	1	1	4	2
N. J. B42	1	1	4[b]	2–3
Ia. I234	1	–	4	3–4
Ia. B1 345	1	–	4	4
Ia. B1 349	1	–	4	4
Ill. A	1	1	4[b]	4
Ill. 4226	1	1	4	4
T. 18C	1	1–2	3	1
T. 10B	1	1–2	3	2
Ia. KB 397	1	1–3	4	3–4
US 3–(S6)–4–1	1	1–4	2	3
Ill. R4	1	2–3	3	3
Ohio 67	1	2	3	2
Ind. Tr.	1–2	–	4	3–4
US 6–(S6)–2	1–2	1–2	3	4
Ind. WF 9	2	1	3	2–3
US 5–(S6)–1–1	2	1	4[b]	4
Ind. B2	2	1	4[b]	2
Ia. I197	2	1	4	3
US 43	2	1–2	4	3
US 62	2	1–2	3	3
Ia. WD 456	2	1–2	4	3
Tx. 158–4	2	1–2	3	2–3
Ohio 56	2	2–3	4	4
Ia. Os420	2–4	1–4	4	3–4
US 7–(S5)–1–1	3	1	3	3
N. J. A64	3	1	4	4
Ill. 90	3	1–2	3	2
US 33(S10)–3	3	1–2	3	2–3
N. J. A12	3	1–2	4	2–3
US 61	3	1–3	3	2–3
Ia. L289	3	1–3	3	3
Ia. L317	3	1–4	2	1
US 2–(S6)–1	3	1–4	3	4
Ia. ITE 701	3	2	4	4
Tx. 102A	3	2	–	3
KYS	5	1–4	5	3

[a] Grade 1 = resistant, and grade 5 = very susceptible.

[b] In some inbreds the record is doubtful. Plants became prematurely dry.

was somewhat more susceptible to seedling inoculation than to late natural infection as leaf blight. Seedling reaction to inoculation apparently is not a safe indication of susceptibility to leaf blight in maturing plants. This is borne out under natural conditions in the field where leaf blight is not only much more abundant but also more destructive than early, primary infection. Leaf-blight ratings are fairly uniform for each strain for the two years, but, on the whole, there was somewhat less leaf blight in 1940 than in 1939.

These differences in the resistance of dent-corn inbreds to bacterial leaf blight indicate promising possibilities for the development of corresponding resistance in dent-corn hybrids. This is of special importance as the use of resistant strains is the only practical means of controlling bacterial leaf blight.

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CONSERVATION OF SCHOLARLY JOURNALS

The American Library Association created this last year the Committee on Aid to Libraries in War Areas, headed by John R. Russell, the Librarian of the University of Rochester. The Committee is faced with numerous serious problems and hopes that American scholars and scientists will be of considerable aid in the solution of one of these problems.

One of the most difficult tasks in library reconstruction after the first World War was that of completing foreign institutional sets of American scholarly, scientific, and technical periodicals. The attempt to avoid a duplication of that situation is now the concern of the Committee.

Many sets of journals will be broken by the financial inability of the institutions to renew subscriptions. As far as possible they will be completed from a stock of periodicals being purchased by the Committee. Many more will have been broken through mail difficulties and loss of shipments, while still other sets will have disappeared in the destruction of libraries. The size of the eventual demand is impossible to estimate, but requests received by the Committee already give evidence that it will be enormous.

With an imminent paper shortage attempts are being made to collect old periodicals for pulp. Fearing this possible reduction in the already limited supply of scholarly and scientific journals, the Committee hopes to enlist the cooperation of subscribers to this journal in preventing the sacrifice of this type of material to the pulp demand. It is scarcely necessary to mention the appreciation of foreign institutions and scholars for this activity.

Questions concerning the project or concerning the value of particular periodicals to the project should be directed to Wayne M. Hartwell, Executive Assistant to the Committee on Aid to Libraries in War Areas, Rush Rhees Library, University of Rochester, Rochester, New York.

LEE ELLIS MILES

September 25, 1890–May 10, 1941

DAVID C. NEAL

Dr. Lee Ellis Miles, Plant Pathologist of the Mississippi Agricultural Experiment Station and Agent of the Division of Cotton and Other Fiber Crops and Diseases, U. S. Department of Agriculture, died suddenly of a heart attack on May 10, 1941, at Greenville, Mississippi. At the time of his death he was collecting field data on cotton and oat diseases in the Mississippi Delta region. He was the son of Charles and Sarah Jane Acres Miles, Irish and Welsh parents, and was born in Rockville, Indiana, September 25, 1890. He was valedictorian of the graduating class of Rockville High School in 1910, and was graduated from Wabash College with the A.B. degree in 1914. For high scholastic ability in his junior year at Wabash, he was elected to Phi Beta Kappa. He was a member of Lamba Chi Alpha social fraternity. Dr. Miles served in the Indiana National Guard in 1914 and, during the World War I, he was attached to the 25th Machine Gunners' Battalion, U. S. Army.

In 1917, the University of Illinois awarded him a fellowship in plant physiology and, in 1920, he received the degree of Ph.D. from the same institution. His doctorate dissertation was "Leaf Spots of the American Elm."

During the summers of 1919–20 he was employed by the Bureau of Plant Industry, U. S. Department of Agriculture, in Barberry eradication and White Pine Blister Rust Control in the States of Illinois, Michigan, Wisconsin, and Tennessee.

From 1920 to 1922, Dr. Miles served as plant pathologist for the Mississippi State Plant Board. He was in charge of eradication of diseases of sweet potatoes, sugar-cane mosaic, etc., plant-disease inspection of nurseries, and was a member of the advisory board on plant-disease control. In 1922, he resigned his position in Mississippi to become pathologist of the Alabama Agricultural Experiment Station and Professor of Plant Pathology at the Alabama Polytechnic Institute, Auburn, Alabama. During his residence in Auburn he was married to Miss Eunice Rebecca Stodghill, who survives him together with two daughters, Lallah Jane and Mary Martha Miles. In 1927, Dr. Miles accepted the position of Assistant Plant Pathologist with the Experiment Station and Assistant Professor of Plant Pathology at State College of Washington, Pullman, Washington. He remained in this position only one year and returned to Mississippi in the summer of 1928, where he served as Plant Pathologist of the Mississippi Agricultural Experiment Station, State College, Mississippi, to the time of his death. At the Mississippi station, he became actively interested in the cotton-disease investigations sponsored by the Cotton Disease Council and conducted in cooperation with the Bureau of Plant Industry, U. S. Department of Agriculture, and made

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LEE ELLIS MILES

several scientific contributions in this field. He investigated particularly the seedling diseases of cotton, cotton seed treatment, the effect of period and type of storage following treatment, and varietal resistance of cotton to fusarium wilt and root-knot nematode. He also was the first to discover verticillium wilt of cotton and downy mildew of oats in Mississippi. He spent the summer of 1940 at Harvard University in taxonomic research on this downy mildew fungus.

He was a student of mycology, two of his noteworthy publications being: "The Rusts of Mississippi," and "The Ascomycetes of Mississippi," published during the years 1933–35. He knew the fungi and their host plants well; and in conducting research in applied plant pathology, he proceeded not only with scientific vision but with the practical aspects of plant-disease control as the uppermost objective. He enjoyed botanical field trips, botanical collecting, and athletic sports (particularly football, basketball, and track), and took an active part in scientific meetings. He served the Southern Section of The American Phytopathological Society as Secretary from 1923–25; President, 1930–31; and as member of the Council of the American Phytopathological Society 1932–33. He was a life member of The American Phytopathological Society, member of the Botanical Society of America, member of the Executive Committee of the Cotton Disease Council, Fellow of American Association for the Advancement of Science, and member of Sigma Xi.

A forceful speaker and endowed with a dry sense of humor, Dr. Miles always commanded attention and respect whenever called upon to make impromptu remarks or to address a scientific meeting or other assemblage. A strong personality was Dr. Miles, devoted to his family, loyal to his friends, and sympathetic and tolerant toward the views of others. He lived a full and fruitful life, which endeared him to a host of friends. Regardless of whether it was the college associate, the merchant, or family doctor who accompanied him on deep-sea fishing trips, the farmer or plant grower who discussed his plant-disease problem with him in the field, or the co-worker who collaborated with him on disease surveys or at scientific meetings, the lives of all of these acquaintances are richer for having known him.

His passing marks a distinct loss to plant pathology, to southern agriculture in particular, and to Mississippi, his adopted State, whose citizens he served with distinction for many years.

Dr. Miles conducted research and published numerous articles in varied fields of plant pathology including diseases of cereals, cotton, nursery and ornamental plants, pecans, truck crops, and sugar cane. A list of his publications together with those of joint authorship follows:

1. Miles, L. E. The leaf spots of the elm. Univ. of Illinois Thesis. Reprinted from Bot. Gaz. 71: 161–198. 1920.
2. ———. Angular leaf spot of cotton in Miss. in 1934. (Abstract) Phytopath. 25: 973. 1935.
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DIFFERENCE IN pH RELATIONS OF SOME PATHOGENICALLY VARIABLE STRAINS OF TOMATO FUSARIUM

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INTRODUCTION

The well-known tomato wilt caused by *Fusarium bulbigenum* var. *lycopersici* (Brushi) Wr. and R., has been an important disease for many years. Strains of tomato that are wilt-resistant under ordinary field conditions have been bred and selected by numerous workers. At times, however, these varieties are more severely damaged by *Fusarium* than might be expected; a fact that may be due in part to the presence of fungus strains of increased virulence. More highly wilt-resistant varieties of tomato are being developed (1, 4), and, in the course of such work done at the U. S. Horticultural Station, Beltsville, Md., studies have been made of the relative virulence of a number of strains of the causal organism.

A considerable range of pathogenicity (11) has been shown to occur in strains[1] of the tomato wilt *Fusarium*, varying from those of mild type, producing little damage, through those causing increased disease, to a highly virulent type. Attention also has been given (12) to the direction and rapidity of variation. While studying variant *Fusarium* strains in culture and pathogenically, contrasts were seen to be so great as to indicate the probability of marked divergence in physiology of the strains *in vitro*. These observations were pursued further, and one phase of the problem included a study of certain pH relationships. The research here reported does not represent an exhaustive study of hydrogen-ion relationships of the tomato-wilt *Fusarium*, since the results were considered first of all as an addition to the data on the divergence of strains of the organism. It is believed, however, that they may also be of interest in connection with the complex problem of relative virulence in variant isolates.

MATERIAL AND METHODS

A total of 79 different isolates or strains of *Fusarium bulbigenum* var. *lycopersici* were used in these studies. One series of 28 strains had been previously studied and described (11) and its members were known as to pathological and cultural histories. They varied pathogenically from extremely virulent through intermediate types to very mild when tested on seedlings of Bonny Best and Marglobe tomato and, culturally, from the class bearing raised, white, woolly mats through intermediate types to the appressed class characterized by dark slimy mats with little or no aerial growth. Of these strains, two were selected for intensive studies on the basis of approximately the widest divergence in pathogenicity, as already determined (11, 12). The most virulent isolate was No. 5; the mildest was No.

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[1] The term "strain" refers to cultural progenies from a given isolate.

15, both of single-spore origin. These were then further "pure lined" by use of monosporic reisolation methods. With both strains, 8 monosporic isolates were made for each "cultural generation." These were grown and tested according to standard technique (10) for pathogenicity. In the case of the R5 isolates, 6 "generations" were grown, and in each generation the culture giving highest pathogenicity was kept for reisolation purposes and the 7 remaining cultures discarded. The same method was followed for 8 generations in the case of the A15 strain, except that the colony from which lowest pathogenicity index was obtained was used for the reisolation purposes. The object of this procedure was selection of an "R" strain on the basis of highest pathogenicity and selection of an "A" strain on the basis of lowest pathogenicity. These were designated separately from their parents, namely, R5–6 and A15–8; and have been compared in detail in table 1.

TABLE 1.—*Comparison of certain characters of the R5–6 (virulent) and A15–8 (mild) strains of Fusarium bulbigenum var. lycopersici*

Points of comparison	Strain R5–6	Strain A15–8
Source of strain	6 monosporic reisolations from R5[a]	8 monosporic reisolations from A15[a]
Culture class and virulence[b]	Raised, virulence index = 15.00	Appressed, virulence index = 1.90
Aerial growth on agar[c]	Abundant, fine woolly	Scanty tufts of hyphae
" " " liquid[d]	Thick, fluffy, floating	Thin, slimy, mostly submerged
Mat texture on agar	Tender, cheesy	Tough, stringy
" " in liquid	Brittle, folded and warty	Tough, smooth
Color[e] of agar culture	White to pale lavender	Vinaceous purple to light buff
Color of rice culture	Pale persian lilac	Cinnamon
Color of liquid culture	White to pale vinaceous lilac	Tawny
Odor of agar culture	Ammoniacal	Sweetish aldehyde
Odor of liquid culture	Strong ammonia	Slightly sour, yeasty

[a] Previously described and studied (11, 12).

[b] Reference is made to prior publications (10, 11, 12) for definition of culture classes and numerical evaluation of virulence. Indexes from parallel tests on 20 plants of Bonny Best tomato: 15.00 = plants dead and collapsed; 1.90 = plants with browned vascular bundles in roots nearly up to cotyledonary node, but externally plants apparently healthy.

[c] Agar of the "differential formula" described in this paper. Grown 2 weeks on all media.

[d] Liquid used was of Tochinai formula (10).

[e] Color according to Ridgway (6).

In addition to the 30 isolates just mentioned, 49 others were secured within 2 summer seasons from field-grown Bonny Best plants that were in various stages of wilt. These latter cultures were single-spore isolates, each from a separate plant, except for 6 saltants out of these, which also were included. These cultures were all found to be well within the limits answering the description by Wollenweber and Reinking (14) of *Fusarium bulbigenum* var. *lycopersici.*

The culture medium generally used was made according to the Tochinai formula (10). As a liquid medium it had a pH of about 6.0 to 6.2. Different reactions were produced by the use of separately prepared and sterilized dilute hydrochloric acid and sodium hydroxide solutions, mixed, after cooling, in equal quantities with double-strength culture medium. It was found that Tochinai agar did not give the most distinctive reactions between different cultural variants. By varying the components of several of the best known of the standard culture media used with fusaria, a formula was finally devised that gave the most rapid and consistent results in differential studies between R5–6 and A15–8, as well as other strains. The formula is as follows: proteose peptone 5.0 g., dihydrogen potassium phosphate 0.5 g., magnesium sulphate 0.5 g., maltose 15.0 g., ferrous sulphate 0.03 g., agar 12.0 g., water 1000.00 cc. As a liquid it had a pH value of about 5.2 to 5.4.

All glassware employed was of the "insoluble" type. Liquid cultures were grown in 100 cc. of liquid in 200 cc. small-mouthed Ehrlenmeyer flasks. Agar cultures were in Petri dishes 9.0 × 1.5 cm. containing approximately 20 cc. of agar. Every pH determination was made with the Beckman pH meter, standardized at frequent intervals against known buffer solutions. Except in a few cases that are mentioned, cultures were held in a dark incubator at an approximate temperature of 27.5° (± 1.2°) C.

Standard-size pieces of inoculum were taken from 21-day-old Tochinai agar plates that had been inoculated in the center. These plates were set in the center of a block on which was drawn a circle the exact diameter of the bottom half of a Petri dish containing agar. Extending at equidistant points around the periphery of the circle were markings to indicate radial lines. A sharp-edged steel tube was employed to cut disks of 5.5 mm. diameter from near center to edge along these radial lines, so that the inoculum for each cultural treatment within a test was taken as nearly as possible along one radius of mat growth. Preliminary studies showed that in apparently stable strains of *Fusarium* this technique resulted in a uniform selection of inoculum. In obviously saltating strains results of saltant action were more certain of demonstration, since it was observed that sectors occurred more frequently along radial lines. When numerous disks for inoculum were cut, supposedly at random, in an agar plate there was apt to be unconscious selection of disks on the basis of ease of handling rather than age of growth or cultural variability.

Every flask of liquid was inoculated with a single disk of inoculum. These were shaken immediately, put in the incubator, and left untouched until the desired period of growth had elapsed. In the case of mixed cultures, a half-disk of one strain matched with the half disk of another was used for inoculum. In agar experiments disks were placed with aerial growth down at the center of Petri plates, on the hardened agar surfaces, care being taken to avoid any sliding of the disk on the medium.

Each datum that refers to either pH readings or mat weights represents the mean of at least 3 determinations. Weights were secured by first pouring the contents of incubated flasks onto weighed, air-dry filter paper in a Buchner funnel attached to a low vacuum water pump. Excess liquid was then withdrawn, mats were washed with 100 cc. of cold water, which was filtered off, and the mat and papers dried at room temperature. This room was steam-heated, and, under the local laboratory conditions, drying proceeded rapidly and approached equilibrium in about 4 days. Final weights, however, were not recorded until after drying 14 to 16 days. To correct for the weight of the inoculum air-dry weights of 9 disks from each place used as source of inoculum were obtained by dropping newly cut disks into liquid medium, shaking, filtering immediately, and drying. The average inoculum weight was subtracted from the final mat weight, so as to indicate growth on the basis of net weight. Growth of cultures was on the whole very uniform, variations being small within triplicates. This also was true of mat areas obtained on agar cultures.

In the case of growth on agar, results were recorded as areas in square centimeter of agar covered. Means were taken in millimeters of 4 radii on each colony grown in a triplicated pH series. From this a "gross" area was calculated from which the mean area of the pieces of inoculum were subtracted.

Disease evaluations were determined (10) on the susceptible and tolerant test hosts, Bonny Best and Marglobe. Numerical indexes are briefly as follows: 0 = no observable internal or external disease symptoms; 1, 2 = mild disease, discolored vasculars in base of plant up to cotyledonary node, no leaf symptoms; 3, 4, 5 = moderate disease, darkened veins in the base of stem, lower leaves wilting; 6, 7 = serious disease, dark vasculars in more than half of stem, lowest leaves wilted, leaves in central portion of plant wilting; 8, 9 = severe disease, dark vasculars extending nearly to tip, almost all leaves wilted except those at tip; 10 = very severe disease, vasculars dark full length of stem, all leaves, even at tip, wilting or dead, stem of plant upright; 11 to 15 = extreme severity, all leaves dead, stem condition ranging from first signs of collapse to complete prostration.

PROGRESSIVE pH CHANGES IN A LIQUID MEDIUM

Parallel sets of flasks of Tochinai liquid having a pH of approximately 6.1 were inoculated with R5-6 and A15–8 and placed in the incubator. Sample sets of 3 cultures were removed from these series at intervals for determinations of change both in pH and in weights of air-dry mats. Data from 5 series were taken at the following intervals: series 1, at 0, 3, 10 and 15 days; series 2, at 0, 10, 15, 20, 30 and 40 days; series 3, at 0, 1, 4, 7, 10, 20 and 32 days; series 4, at 0, 5, 12, 19, 50, 100, 120 and 150 days; and series 5, at 0, 3, 5, 8, 12, 16, 22, 27, 33, 41, 53, 60 and 67 days.

From what had been learned from previous series, the fifth was designed so that samples were taken at the best intervals to demonstrate as complete details as practicable of the critical pH and weight changes. Data from the other series differed so little from No. 5 that the latter may be taken as characteristic of all the series. Results from it are therefore the only ones to be given and are represented in figure 1, A and B.

It will be seen from the curves representing weight changes (Fig. 1, B) that the virulent strain produced much greater mat weight than did the mild strain, and the high point was gained by the virulent strain at around 12 days' growth. It took the mild strain 22 days to reach its greatest

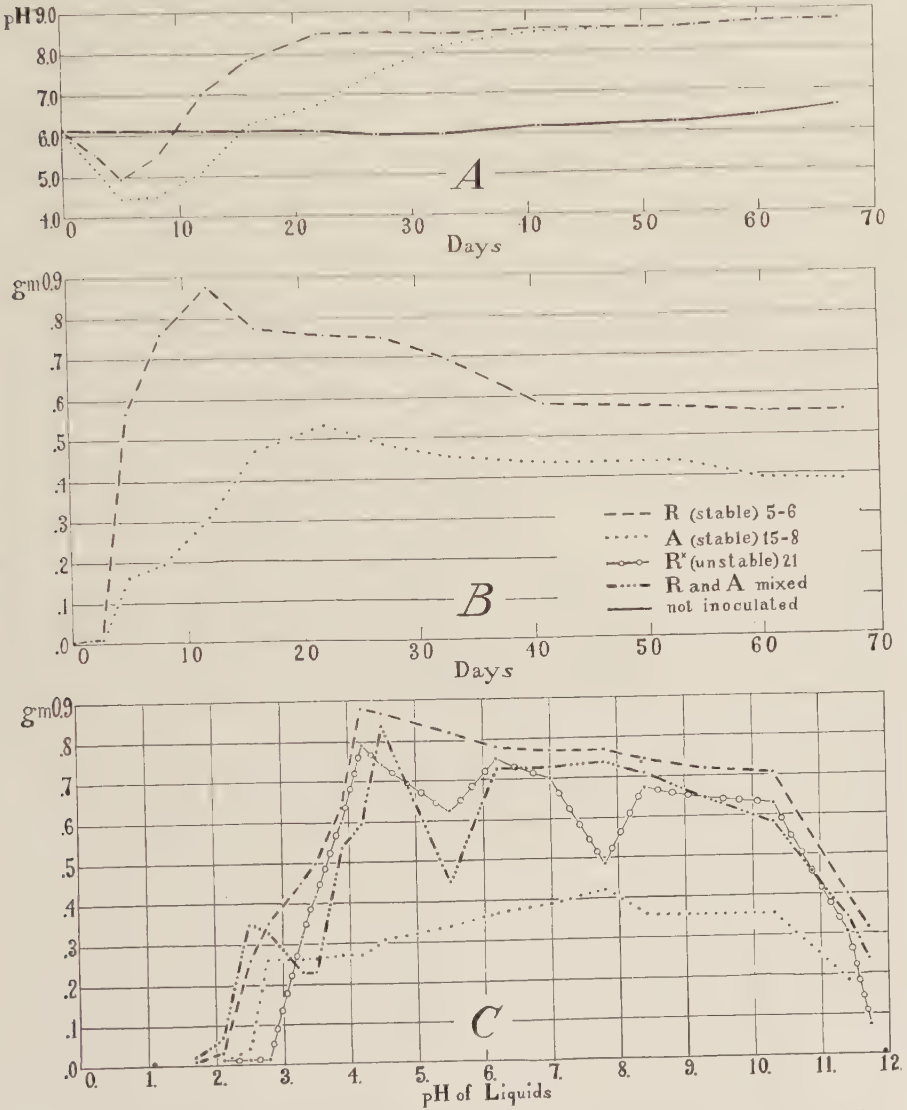

FIG. 1. Growth in liquid cultures (Tochinai formula) of pathogenically different strains of *Fusarium bulbigenum* var. *lycopersici*. A. Progressive changes in pH produced by a virulent (R5–6), and a mild (A15–8), strain of the organism. B. Progressive mat-weight determinations for the virulent and mild strains. These data taken concurrently with A and from the same cultures as those tested for pH. C. Mat weights produced after 16 days' growth in media initially adjusted to a wide range of pH.

weight. After reaching their respective high points, both strains lost some weight, apparently because of partial disintegration, or autolysis, of the mycelial mats. This loss was arrested finally, as the curves indicate. It will be noted in figure 1, A, that immediately after either of the strains began growing acidity increased in the liquids for the first 5 days, and was most intense in cultures of the mild organism. In these cultures the pH then rose gradually until about the 40th day. It took the virulent culture about half the time required by the mild organism to go through the same course of changes.

It is interesting also to observe differences in the concurrent pH and weight changes produced by the A and R strains (Fig. 1, comparing curves shown in diagrams A and B). For R5–6, by far the greatest increase in mat weight was at the beginning of growth between 2½ and 5 days after inoculation, during which time the liquid was becoming most acid. For A15–8, on the other hand, a comparatively rapid increase in mat weight continued in progress for around 15 days after growth in culture was well established. During this time the liquid attained its greatest acidity, then it became less acid until it approached neutrality. It required about 40 days for R5–6 cultures to reach a point where little further loss in mat weight occurred, and about 60 days for the A15–8 cultures.

GROWTH AFFECTED BY DIFFERENT pH REACTIONS OF MEDIA

Agar Medium

It should be repeated here that no attempt was made to execute a complete study of the relation of pH ranges in media to growth of strains of the tomato-wilt *Fusarium*. The main purpose was the demonstration of differences in the physiology of strains of the pathogen.

In figure 2 are represented smoothed curves giving the comparison between areas covered after 115 hours of growth at 27.5° C. on agars of different initial pH reactions by the virulent and mild strains. These data are the means of results from 3 experiments that were run within a few days of each other, and for the same number of hours in the same temperature chamber. The agars in each (handled in triplicate) were adjusted to 12 pH points from 2.2 to 11.7. Except in a very few instances in the extremely acid or alkaline agars, the curves for the individual experiments ran close to and did not cross the smoothed curves depicted in figure 2.

On agar the optimum pH ranges were much alike for strains A15–8 and R5–6. The greatest areas of growth were covered with both strains between a pH of 7.0 and 8.7. The greatest difference between these cultures on agar was seen in the comparative amounts of growth produced. Nearly 20 per cent more area was covered by the mild strain of the organism than by the virulent strain.

Liquid Medium

Studies were likewise made of mat weights produced in liquid medium adjusted to different pH readings. Results of typical experiments are represented in figure 1, C, and the data from the other series are given in tables 2, 3, and 4. There were slight divergences, but, on the whole, the virulent

R5–6 strain had an optimum at the quite acid reaction of approximately pH 4.0, while the mild A15–8 had an optimum at a definitely alkaline reaction of about pH 8.0. Extremely acid (pH 2.0 to 3.0) and extremely alkaline (pH 11.0 to 12.0) media were unfavorable to about an equal degree for both strains of the organism. When mat weights from either of the stable strains, A15–8 or R5–6, were plotted against original pH reactions of media (Fig. 1, C) the results were comparatively regular curves having single optimum points. When the data from unstable strains were handled in the same way, the curves showed considerable irregularity with more than one point of optimum growth.

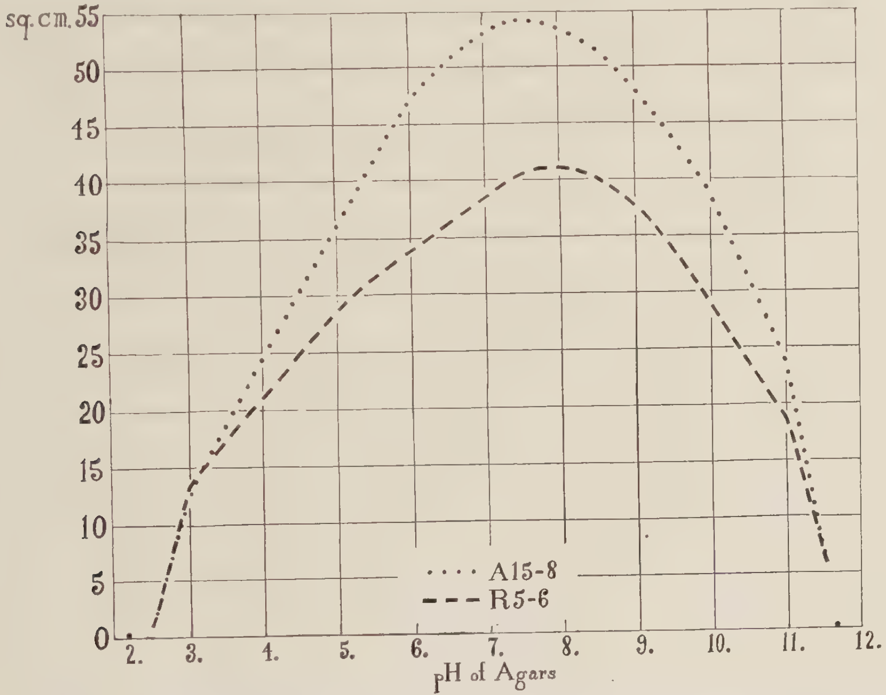

FIG. 2. Areas covered by a virulent and a mild strain of the tomato-wilt *Fusarium* on agar (Tochinai formula), adjusted at 12 points over a wide range of pH. The smoothed curves represent the mean areas covered at these points on 9 agar cultures from 3 separate series. Each series was grown 115 hours at 27.5° C.

Equal-size bits of mycelium from A15–8 and R5–6 were combined and used to inoculate a total of 4 culture series that had wide ranges of pH. After incubation, mat weights were obtained from these series, and the results (Fig. 1, C, and Table 3) gave an indication of the occurrence of more than one maximum growth point with considerably reduced growth between these rises. The general tendency seemed to be for two such maxima to develop. In addition to this tendency, seen among tests in the series inoculated with mixtures of the stable strain and mild strain, what appeared to be bimodality was found in all of 7 tests of cultures from 3 strains of the *Fusarium* known to be unstable and that were distinctly saltating at the time they were used for inoculum. The significance of these findings in comparison with the findings of other workers on the same organism, will be examined below in the discussion.

TABLE 2.—*Growth*[a] *in Tochinai liquid medium of virulent (R5–6) and mild (A15–8) strains of Fusarium bulbigenum var. lycopersici as influenced by various initial pH reactions*[b] *of the medium*[c]

Series 1[d]			Series 3[d]		
Reaction of medium	Average mat weights		Reaction of medium	Average mat weights	
	R5–6	A15–8		R5–6	A15–8
pH	*Gm.*	*Gm.*	*pH*	*Gm.*	*Gm.*
1.0	.00	.00	3.1	.27	.17
1.7	.01	.00	3.2	.38	.18
2.1	.03	.01	3.6	.50	.18
2.8	.35	.26	4.2	**.56**	.20
3.3	.49	.27	4.7	**.57**	.21
3.8	.65	.25	6.0	**.53**	.23
4.1	**.89**	.27	6.7	**.53**	.25
4.3	**.87**	.30	7.2	.50	.29
5.2	**.83**	.29	7.7	.50	**.32**
6.1	.70	.31	8.1	.49	**.32**
6.8	.70	.34	8.4	.46	.26
7.8	.71	**.40**	9.1	.41	.24
8.6	.71	**.37**	9.7	.37	.25
10.2	.68	.35			
11.2	.41	.19			
11.7	.30	.01			
12.0	.00	.00			

[a] Growth recorded as the average weight of 3 fungal mats.

[b] Reactions regulated through use of dilute HCl and NaOH.

[c] Tochinai liquid (10).

[d] In these experiments 4 series were run: Data from series 2 are represented in figure 1, C; from series 4 presented in table 3. All series incubated in the dark at approximately 27.5° C., series 1 and 2 for 14 days; series 3 for 9 days; series 4 for 16 days.

CHANGE IN REACTION OF LIQUID CULTURES STARTED AT DIFFERENT pH LEVELS

Changes in pH of liquid media having different initial reactions were studied in parallel series inoculated with the virulent R5–6 and the mild A15–8 forms. There were 4 experiments in these studies, and in each one variations were seen between the growth changes of the mild and virulent organisms, as is shown in the data of table 4. The general tendency was for the virulent strain to reduce acidity of the originally acid liquids in which, at the same time, it had produced its best growth. The pH of the acid liquids was changed but very little by the mild strain. On the whole, liquids with reactions around neutrality were made more alkaline by the virulent strain; comparatively, they were changed less by the mild strain. The liquids that were initially of moderate alkalinity in which the mild strain produced its maximum growth were at first made more acid by its growth and changed but little by the virulent strain. These mild cultures became slightly more alkaline than the original pH level as their age increased, while the virulent cultures displayed a greater degree of alkalinity.

TABLE 3.—*Growth*[a] *in Tochinai liquid medium of different strains of Fusarium bulbigenum var. lycopersici as influenced by various pH reactions of the medium*

Reaction of medium when inoculated	Weight produced by the Fusarium strains indicated										
	R5–6[b]	A15–8[c]	R5–6 and A15–8 mixed			RS 29[d]			IR 13[e]		
	Series 4 Test No.	Series 4 Test No.	Series 1 Test No.			Series 1 Test No.			Series 1 Test No.		
	1	1	1	2	3	1	2	3	1	2	3
pH	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1.7	.01	.00	.02	.01	.01	.02	.01	.00	.01	.01	.01
2.1	.03	.01	.06	.04	.05	.03	.03	.04	.03	.03	.03
2.5	.23	.04	.36	.35	.16	.09	.14	.07	.14	.06	.08
2.9	.35	.26	.33	.23	.29	.15	.14	.20	.21	.22	.17
3.2	.49	......	.23	.40	.41	.22	.25	.24	.20	.22	.09
3.5	.46	.26	.45	.46	.36	.25	.20	.22	.17	.16	.17
3.8	.65	.27	.54	.46	.49	.27	.55	.50	.15	.19	.22
4.2	.89	.27	.84	.80	.84	.40	.31	.35	.22	.20	.20
4.5	.87	.31	.87	.80	.45	.36	.38	.33	.16	.22	.21
6.1	.77	.37	.40	.81	.35	.36	.37	.45	.17	.19	.22
7.1	.71	.41	.73	.80	.71	.33	.39	.37	.21	.25	.28
7.7	.74	.37	.74	.64	.73	.41	.42	.39	.24	.24	.23
8.4	.61	.36	.72	.69	.72	.42	.44	.40	.22	.25	.22
10.3	.51	.36	.56	.55	.58	.....	.40	.35	.25	.25	.31
11.4	.41	.19	.35	.38	.27	.27	.23	.29	.10	.15	.22
11.7	.30	.00	.24	.29	.25	.12	.14	.14	.12	.00	.00

[a] Growth recorded as the mean of air-dry weights of mats from 3 cultures in each treatment. Cultures incubated 16 days in the dark at approximately 27.5° C.

[b] Stable, pure-line, highly virulent strain. See table 1 and text for description.

[c] Stable, pure-line, very mild pathogenically. See table 1 and text for description.

[d] Unstable, vigorously saltating strain, severe at times but erratic in its pathogenic behavior (11).

[e] Unstable, saltating strain, of medium somewhat erratic pathogenicity (11).

CHANGE IN pH OF LIQUID CULTURES INDICATIVE OF RELATIVE PATHOGENICITY

Isolates of Known Pathogenicity

As the curves of progressive pH change by the highly virulent and very mild strains of the organism were studied, it seemed probable that comparative degrees of acidity developed in liquid cultures of isolates unknown as to disease capacity might indicate their relative pathogenicities. It can be seen from reference to the curves presented in figure 1, A, that in R5–6 and A15–8 the greatest spread between pH readings occurred during the third week of incubation. Cultures of this approximate age, therefore, were tested for acidity in these studies.

The first group of cultures used were the 28 isolates that had previously been studied (11) for relative pathogenicities correlated with culture classes. All isolates were grown on the differential medium described above under "Materials and Methods," they were checked as to appearance, and cultures 3 weeks old were used as source of inoculum for triplicate sets of flasks of liquid medium. A number of representative members in each of the 5 culture classes were used for pH tests after 9 days incubation, and all of

TABLE 4.—*Changes in reaction of culture liquids when inoculated at different initial pH levels*[a] *using the virulent R5–6, and the mildly pathogenic A15–8 strains of Fusarium bulbigenum var. lycopersici and allowed to grow for various periods*[b]

Experiment 1[c] Incubated 9 days			Experiment 2 Incubated 12 days			Experiment 3[d] Incubated 16 days			Experiment 4 Incubated 19 days		
Original reading	After growth		Original reading	After growth		Original reading	After growth		Original reading	After growth	
	R5–6	A15–8		R5–6	A15–8		R5–6	A15–8		R5–6	A15–8
pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
......	......	......	......	......	......	2.9	2.8	2.7	2.7	2.3	2.6
3.2	3.5	3.2	3.2	3.7	3.2	3.2	3.5	......	3.0	4.2	3.2
3.6	4.1	3.8	......	......	......	3.5	4.7	3.6	3.2	7.6	4.0
......	......	......	......	......	......	3.8	5.2	4.3	3.6	8.3	4.9
4.2	4.7*[e]	4.7	4.3	7.3*	4.8	4.2	6.9*	4.4	4.0	8.3*	5.6
......	......	......	......	......	......	4.5	7.6*	4.9	4.3	8.3*	6.4
4.7	6.7*	5.2	......	......	......	......	......	......	4.6	8.4*	6.6
......	......	......	5.1	8.0	5.2	......	......	......	5.1	8.5	6.8
6.0	7.3	5.3	6.1	8.0	5.6	6.1	7.5	6.0*	6.1	8.5	7.7*
6.7	8.0	5.6	......	......	......	......	......	......	6.6	8.6	7.7*
7.2	8.1	6.2	......	......	......	7.1	7.5	......*	......	......	......
......	......	......	......	......	......	......	......	......	7.4	8.6	7.9*
7.7	7.9	6.7*	7.8	8.1	6.1*	7.7	7.5	6.5*	......	......	......
8.1	7.9	6.7*	8.2	8.1	6.3*	......	......	......	8.2	8.8	7.9*
8.4	7.9	7.0	8.6	8.2	6.3*	8.4	7.6	6.9	......	......	......
......	......	......	8.8	7.8	6.5	......	......	......	8.8	8.8	8.1
......	......	......	9.0	7.4	6.8	......	......	......	9.1	8.8	8.2
......	......	......	......	......	......	......	......	......	9.6	8.8	8.2
......	......	......	......	......	......	......	......	......	9.8	8.8	8.5

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[a] Liquids of Tochinai formula, regulated to different pH points (original readings) by using dilute HCl and NaOH solutions. Data not included from extremely acid or alkaline liquids in which growth was absent or much reduced.

[b] The 9- and 16-day series incubated in the dark at approximately 27.5° C. Others at irregular room temperatures.

[c] Data from the same experiment as series 3, table 2.

[d] Data from the same experiment as series 4, table 3.

[e] Asterisks (*) denote most vigorous appearing mats in culture at end of incubation period. That such appearance is a correct estimate of the maximum mat development may be seen by comparing weights of series 3, table 2, with data in experiment 1 above, also series 4, table 3, with experiment 3 above. The actual mat weights were secured from the liquids tested for pH in experiments 1 and 3.

the strains were tested after growing 18 days (Table 5). For points of reference it should be kept in mind that No. 5 was the "parent" for the pure-line strain R5–6 used in other parts of these studies, and No. 15 was the "parent" for the pure-line strain A15–8.

It may be seen that after 9 days there was indication that the most highly pathogenic cultures were closer to neutrality than the milder strains, which were slightly on the acid side. When a total of 18 days had passed, differences in pH were even more notable: high virulence was accompanied by increased alkalinity; the mildest strains were most acid in reaction; and with the intermediate strains, the pH lay between the extremes.

TABLE 5.—*Change in pH of liquid medium*[a] *due to growth of isolates of tomato wilt Fusarium that had been previously studied for relative pathogenicities*[b]

Isolate	Culture class	Reactions of inoculated liquids	
		9 days incubation[c]	18 days incubation[c]
No.		*pH*	*pH*
8	Raised	......	8.2
5	‘‘	6.2	8.3
21	‘‘	......	8.3
17	‘‘	6.3	8.2
7	Raised sclerotial	......	7.8
9	‘‘ ‘‘	......	7.8
4	‘‘ ‘‘	6.3	7.9
12	‘‘ ‘‘	6.3	8.1
16	‘‘ ‘‘	6.2	8.1
2	‘‘ ‘‘	......	7.8
29	‘‘ ‘‘	......	8.1
11	Intermediate raised	6.2	7.6
26	‘‘ ‘‘	......	7.4
13	‘‘ ‘‘	5.3	7.7
23	‘‘ ‘‘	6.2	7.2
20	‘‘ ‘‘	......	7.3
14	‘‘ ‘‘	......	7.3
3	Intermediate appressed	5.6	7.3
28	‘‘ ‘‘	5.4	7.2
22	‘‘ ‘‘	......	7.2
10	‘‘ ‘‘	......	7.2
19	‘‘ ‘‘	5.5	7.6
18	Appressed	......	6.5
27	‘‘	......	6.9
6	‘‘	4.9	6.9
1	‘‘	5.0	6.3
24	‘‘	5.5	6.0
15	‘‘	4.8	5.5
	Sterile liquid	6.0	6.1

[a] Tochinai medium, formula given elsewhere (10); initial pH value, 6.0.

[b] Arranged according to disease evaluations as reported in a previous paper (11, p. 14); most virulent at top, in descending order of pathogenicity with mildest at bottom. Nos. 25 and 30 not included since they were anomalous members of the 30 isolates tested. It should be noted that later work has shown that No. 5 is slightly more pathogenic and stable than No. 8.

[c] Incubated in the dark at approximately 27.5° C.

ISOLATES OF UNKNOWN PATHOGENICITY

Stems of wilted tomatoes were collected in 5 gardens and fields at a distance from any artificially infested experimental plot. In all, 35 single-spore isolates were secured, 29 of them representing separate isolations from plants and 6 of them appressed saltants from raised sclerotial culture types. All were grown on the special differential agar medium. They were first classed according to cultural types and then used to inoculate flasks of Tochinai liquid medium. The pH readings of the liquids were made after cultures had been incubated from 14 to 16 days. Results were as follows: the 9 raised cultures varied in pH from 7.5 to 8.2 with a mean of 7.9; the 3 raised sclerotial types varied from 7.5 to 7.8 with a mean of 7.7; the intermediate raised varied from 5.9 to 7.6 with a mean of 6.8; the 4 intermediate appressed varied from 6.1 to 6.9 with a mean of 6.6; and the 12 appressed varied from 5.5 to 6.3 with a mean of 5.8.

Another series of 14 cultures of wilt *Fusarium*, unknown as to relative pathogenicities, were studied (Table 6). These consisted of monosporic isolates from separate wilted plants from a naturally infested field. The isolates were grown on the differential agar medium and first grouped according to culture classes. They were then used to inoculate flasks of liquid and after 18 days the cultures were tested for pH values. Disease index (relative pathogenicity) was secured through the use of standard technique (10) on the susceptible host, Bonny Best and the tolerant Marglobe.

TABLE 6.—*Monosporic tomato wilt Fusarium isolates from naturally infected plants, compared with respect to: culture class,*[a] *change in pH of medium,*[b] *and pathogenic evaluation*[c]

Isolate	Culture class	Reaction of liquid	Disease index from host	
			Bonny Best	Marglobe
			Mean value[d]	Mean value[d]
No.		*pH*		
32	R	8.3	9.76 ± 0.77	6.16 ± 0.30
33	R	8.3	11.84 ± .81	6.56 ± .36
40	R	8.1	10.16 ± .86	5.68 ± .36
34	RS	7.9	7.28 ± .49	5.88 ± .63
37	RS	8.1	12.72 ± .64	5.76 ± .42
39	RS	8.0	8.04 ± .68	4.40 ± .26
35	IR	6.7	9.60 ± .66	7.16 ± .36
36	IR	7.4	5.88 ± .36	3.64 ± .02
38	IR	6.6	8.24 ± .69	4.36 ± .29
44	IA	7.1	6.04 ± .30	3.48 ± .02
43	IA	7.3	7.00 ± .30	4.08 ± .24
31	IA	6.7	6.76 ± .38	5.32 ± .38
41	A	5.9	5.80 ± .39	3.20 ± .23
42	A	5.8	5.44 ± .40	4.08 ± .01
R5–6[e]	R	8.3	13.72 ± .52	9.48 ± .54
A15–8[e]	A	5.9	0.68 ± .02	0.48 ± .02

[a] Classes defined and illustrated in other reports (11, 12).

[b] Tochinai liquid (10) used, original pH 6.1, flasks incubated 18 days in the dark at 27.5° ± 1.6° C.

[c] Data from 20 plants in each case. Standard technique (10) used to obtain disease evaluations.

[d] Includes arithmetical means and standard error.

[e] Included for reference purposes.

The isolates in the R class produced strongly alkaline pH reactions of from 8.1 to 8.3, and pathogenicity indexes were uniformly high. The RS-class isolates produced pH reactions from 7.9 to 8.1 with high but irregular pathogenicities. Such irregularities have been noted already (11, 12) as characteristic of the RS culture class. In both the IR- and IA-class groups, pH reactions were from 6.6 to 7.4, all so close to the neutral point as to suggest little variation between cultures. In these data, however, there does seem to be an indication that the IR cultures were slightly more pathogenic than the IA cultures. In the two A-class cultures pH reactions showed considerable acidity, being 5.9 and 5.8 and in these the pathogenicities were the lowest of any of the unknown cultures.

Finally, it is of special interest to compare these data with those obtained on the two known strains, R5–6, which had been particularly studied and selected for its high pathogenicity, and A15–8, studied and selected for its mild disease effects: R5–6 produced a pH of 8.3 equal to the highest produced by any R or RS culture, and it had a pathogenicity on either Bonny Best or Marglobe that was slightly higher than the comparable reaction of any other strain of the organism; A15–8 produced a pH of 5.9 that was similar to the reaction noted for other A cultures, and it had much the lowest pathogenicities on both Bonny Best and Marglobe. The other A strains had pathogenicity indexes that, while they were low when compared with other strains, were at the same time considerably greater than A15–8.

In general these data indicate that differences are unquestionable between R and A cultures as regards appearance, changes produced in pH, and pathogenicities; the two intermediate types (IR and IA), as their names and cultural characters signify, are definitely between the extremes exhibited by the organisms that develop high pH and pathogenicity and low pH and pathogenicity; the intermediate types would appear to be significantly different from the A group on the one hand and either the R or RS group on the other.

DISCUSSION

Where certain phases of the work just presented have necessarily paralleled the investigations of others, *i.e.*, Sherwood (9), Scott (7), White (13), Pritham and Anderson (5), and Luz (3), comparisons of results show that they have not differed greatly beyond what might be expected, considering the variations in conditions and materials represented.

The general shapes of the curves depicting change in mat weight and correlative variation in pH of culture medium over a period of time (Fig. 1, A and B) is in general agreement with similar findings reported by Luz (3). His curves were less regular than these, which indicates the probability that he used an isolate more unstable in growth character than either strain A15–8 or R5–6. Luz also noted the same reduction in pH of medium occurring during the first few days of growth as are indicated in figure 1, A. Pritham and Anderson (5) reported that they did not see such a change. In the present case this reduction in pH was observed a number of times and was always readily determined if the tests were made early enough in the incubation period. Neither Luz nor Pritham and Anderson grew cultures that had end reactions (about pH 7.5 and 7.3) that were quite as high as are indicated in figure 1, A (pH 8.7). This is not to be wondered at, however, for, in neither case, did the other experimenters work under the same conditions or with exactly the same materials as those of the present studies.

Several workers have found that the tomato *Fusarium* produced maximum amounts of growth at two separate points of H-ion concentration, with a considerably smaller amount between these points. Such results have led to the term "bimodal phenomenon" in this connection. This was believed by Scott (8) to be attributable to the isoelectric point of the fungus mycelium, which he found was about pH 5.4. There has been considerable divergence in results among workers with regard to the pH value at which the lowered point between two maxima of growth occurred: Sherwood (9) noted in one experiment that the maxima were on either side of a minimum of pH 7.8 to 8.2; in another experiment this minimum came at 6.6 to 7.0; Scott (7) concluded after a number of experiments that the organism produced one maximum at about pH 4.5 to 5.3, a much reduced growth at 5.3 to 5.8, and a second maximum at about 5.9 to 6.9; White (13) said "all strains showed a double maximum," the first maximum varying with strains, but most generally at 4.0 to 5.5 "and was followed by a minimum at pH 5 to 7, with the second maximum at a point above pH 7"; Pritham and Anderson (5) presented a curve that showed two maximum points with between them a reduction of growth at a little above pH 7.0.

In the results here presented (Fig. 1, and Tables 2, 3 and 4), no "double maximum" was demonstrable, if relatively stable strains (A15–8 and R5–6) were studied separately in culture series. When, however, these were mixed and used as inoculum, striking irregularities in mat weights developed, which might well be interpreted as of "bimodal" nature, and perhaps even typical of the organism, were it not known that the inoculum consisted of a mixture of strains differing in cultural characteristics. In addition, data from a number of other cultures from unstable strains resulted likewise in marked irregularities, due apparently to saltations, that would fit well into the bimodality concept. Reductions in growth, so-called minimum points between maxima, were found in the present studies at around the following pH reactions: 4.2, 4.5, 4.5, 5.5, 6.1, 6.1, 6.1, 7.1, 7.7, 7.7, and 7.7 to 8.4, and such variation compares well with the differing reports of others mentioned above. Reports of double maxima may signify that the experimenter has used a culture influenced by saltant action, which would in effect have been, therefore, a mixed culture. It also appears that the point of reduced growth between two maxima does not necessarily occur at the organism's isoelectric point defined by Scott (8).

White (13) experimented with pH effects on growth of 24 tomato *Fusarium* strains, and attempted to correlate cultural with pathogenic differences. His work is not strictly comparable to that here presented, since he described the characteristics of his cultures on agar slants in test tubes 14 months after inoculation. It has been shown (11, 12) that only by continual vigilance, with cultures in Petri dishes, and by repeated reselection at fairly short intervals, is it possible to be certain of maintaining continuity of growth class in the cultural history of isolates. Recently refinements have been developed (10) in the technique used for testing relative pathogenicities of tomato *Fusarium* strains. White's one experiment along this line was not conclusive, as he himself indicated, and he found it difficult to correlate results of cultural studies with pathogenicity findings. In view of this and the later findings of Haymaker (2), and White's own descriptions, it is fairly probable that many if not all the strains White worked with were actively varying, although this condition may not have been recognized.

The studies herein reported add another method of indicating variation among strains of the tomato wilt *Fusarium*. Along with other growth reactions, isolates may be grown in Tochinai liquid at about 27.5° C. for a little more than 2 weeks, and then tested for pH. Judging from these findings on pH reactions, the highest will be produced by the most virulent cultures, the lowest by the mildest cultures, and those of intermediate virulence will show intermediate pH values. In such tests it is necessary to use standard, known, highly virulent, very mild, and intermediately pathogenic strains along with the unknown cultures for comparison and evaluation of the data obtained.

The investigator might well expect that a dark-colored, appressed, and slimy culture from one source would be very different in physiological behavior from a white, woolly culture from another source. Such difference has been demonstrated in the case of varying cultures, both from numerous separate sources and from only one source. It is of special interest, moreover, when it is recalled that appressed types are readily obtained from originally raised cultures, and that significantly higher raised types may be secured from appressed cultures (12).

From data given in the present paper it is far from possible to know what is the basis for the higher virulence of *Fusarium* cultures of the raised type in comparison with the mildly pathogenic appressed type. Any conclusions as to whether virulence is correlated with either inhibition or more vigorous growth in culture must await further study. At present, it would depend upon the criterion selected for comparison. For example: in liquids the virulent R5–6 strain produced much greater mat weight than the mild strain A15–8. The heavier growth by the virulent organism was quickly followed, however, by rapid loss in weight, probably because of autolysis. The mild strain, after reaching its greatest mat weight, was comparatively less affected by losses resulting from autolysis. On the other hand, on agar surfaces the mild strain covered significantly larger areas than did the virulent one.

SUMMARY

Strains of the tomato-wilt *Fusarium* that differ in pathogenicity are also different in their physiology, as indicated in certain pH relationships and relative amounts of growth.

In liquid culture a highly virulent strain produced its maximum weight in 12 days, while a mild strain required nearly twice this period. The former produced the greater weight in liquid, but, on agar, the mild strain covered the greater area.

In liquid media the maximum growth of the virulent strain was produced only at a pH of around 4.0, and of the mild strain only around 8.0. On agars both had approximately equal maximum points at slightly above neutral. In series of liquid media having a wide range of pH reactions, cultures inoculated with mixed mild and virulent strains produced maxima at two points, as did cultures inoculated with actively saltating, unstable strains. The virulent and mild strains changed the original pH reactions in different degree.

When the culture liquids of a large number of isolates were tested for pH reactions and the pathogenicities of the organisms were determined, it was evident that under parallel conditions, virulent strains produced most alkalinity, mild ones most acidity, and those of intermediate pathogenicity developed reactions between the extremes.

This paper also reports a new differential agar medium by which it was possible to separate with greater ease the variant culture types of the tomato-wilt *Fusarium* strains used in these studies.

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THE EFFECT OF TOBACCO-MOSAIC VIRUS ON CELLULAR RESPIRATION[1]

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INTRODUCTION

Viruses apparently do not respire when removed from contact with living suscept cells (1, 5). Whether viruses exhibit respiratory phenomena during their multiplication in living cells is not known. There is evidence, however, that multiplication of tobacco-mosaic virus protein (*Marmor tabaci* Holmes) may be dependent on cyanide-sensitive respiratory catalysts of the cell (21).

Viruses may represent the extreme in parasitic adaptation of organisms that have become dependent upon their hosts for essentially all metabolic functions, including respiration. On the other hand, it is also possible that viruses represent more or less altered proteins of "host" origin that are capable of some form of multiplication in various "susceptible" species. The source of energy for synthesis of virus proteins is an important consideration regardless of the view concerning their ultimate origin. One approach to this problem is through a study of the relation of the virus to the specific respiratory systems of the cell. This paper presents data from such investigations.

From the available literature it is not possible to determine whether the action of the virus on the respiratory systems consists of a primary chemical action or whether it is due to secondary morphological or physiological changes in the affected organ.

Comparisons of respiratory activity in healthy and diseased tissues that involve samples from different individuals, or different leaves from the same plant, may lead to erroneous conclusions in this respect because of the wide variability in respiratory characteristics that often occurs in such cases. In the present study by use of half-leaf comparison techniques, variations due to genetic, morphological, or physiological differences between control and experimental tissues were practically eliminated. Approximately equal numbers of cells in the diseased and control samples were assured by the use of leaves that had either ceased or almost ceased growing. With these techniques it has been possible to demonstrate that tobacco-mosaic virus affects respiration both quantitatively and qualitatively, influencing the activity of particular respiratory components in a definite time sequence following inoculation. These changes have been followed in single cells as well as in larger masses of tissue.

MATERIALS AND METHODS

A single lesion strain of strongly mottling *Marmor tabaci* was used in all experiments, unless otherwise noted. In some experiments a "yellow" mutant derived from the parent "green" strain was used. Inoculations were made by rubbing halves of fully expanded *Nicotiana tabacum* Turkish leaves with a cheesecloth-covered spatula dipped in freshly extracted infective juice. The opposite half of the same leaf was similarly rubbed with a moistened sterile spatula to simulate the wounding injury of inoculation. Vigorous pot- or bench-grown plants were used in all experiments. Inoculations were made when the plants were 12 to 24 inches high but before development of flower buds.

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[1] Scientific Paper A1. Contribution No. 1717 of the Maryland Agricultural Experiment Station (Department of Botany).

Leaf samples with an area of 1 sq. cm. were removed for respirometer measurements with a steel die. Only tissue lying between the secondary veins was used. Measurements of protoplasmic streaming were made in marginal epidermal cells of the leaf blade. With these techniques it was possible to measure oxygen uptake and protoplasmic streaming in the same leaf. One to 3 mm. of the mesophyll was always included in the sample to insulate the marginal epidermal cells from the injury attendant to cutting out the sample. These marginal cells are ideally suited to measurements of streaming, as they can readily be viewed under the oil-immersion lens, and present a large surface to the irrigating fluid from which they derive their oxygen supply. In measuring cyclotic rate it is extremely important to accurately control the rate of solution flow and to keep the tissue under generally uniform conditions. The cells under observation must be held firmly in place, be freely bathed with the irrigating solution, and not be subject to mechanical injury. The apparatus developed by Aston[2] (unpublished) was found ideally suited to this work and was used in all experiments reported here. Single cells were often kept under observation and in an active state of cyclosis for as long as 4 days.

Measurements of virus concentration in experimental samples were made either immediately following their removal from the respirometers or the samples were stored at 3° C. for several hours before testing. The respirometer samples, after measurement of respiration, were ground up in water, then diluted (about 120 mg. fresh tissue per 3 cc. of water) and the extract rubbed onto half-leaves of *Nicotiana glutinosa* or hybrids of it. The opposite halves of the same leaves were rubbed either with a similarly prepared extract from the control tissue or compared with a standard virus preparation (a similar dilution made from fully invaded Turkish tobacco leaves). The number of necrotic lesions developed on the leaves of these hybrids was used as a measure of virus concentration in the samples.

Oxygen uptake was measured in a polarographic microrespirometer, the tissue being immersed in a ground solution, as previously described by one of us (8) or in air in a modified Fenn (12) differential volumeter (each vessel 7.5 cc. capacity). In the former, significant determinations of oxygen-uptake could be made every 3 minutes on approximately 20 mg. of green leaf

[2] The authors wish to express their appreciation of valuable assistance rendered by Mr. Arthur Aston in design and construction of apparatus used in measuring protoplasmic streaming.

tissue (1 sq. cm.). Five to 7 similar samples were required to give significant measurements every 3 minutes in the Fenn respirometer. The formula for pressure and temperature correction, as given by Fenn (12, pp. 7–8), was used in computing the actual oxygen uptake. Control and infected tissues were run simultaneously, i.e., allowed to "pull against each other." In application of the S.T.P. correction formula the slight error (less than 1 per cent) resulting from reduction in pressure in the comparison volumeter due to respired oxygen was ignored. Oxygen-uptake measurements were made with the tissue in complete darkness to prevent evolution of oxygen in photosynthesis. Rates of oxygen consumption are expressed in *micro liters per hour.*

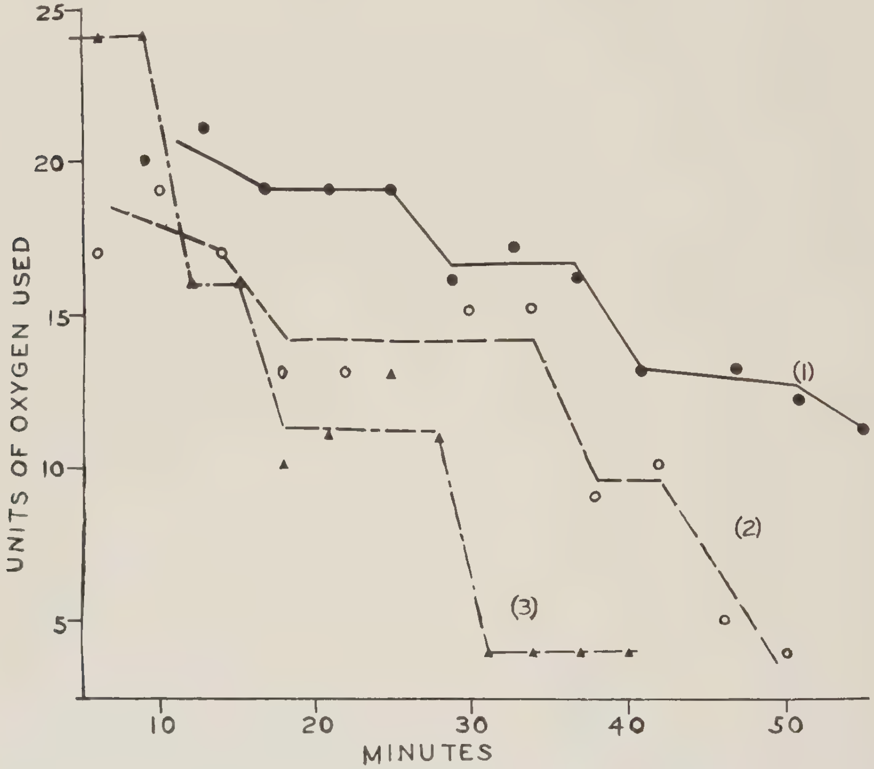

FIG. 1. Relative rates of oxygen-uptake by healthy tissues of three different full-grown Turkish tobacco leaves as measured in the polarographic microrespirometer.

EXPERIMENTAL

Respiration Systems in the Healthy Cell

Measurements in the Polarographic Respirometer. If measurements are made in the polarographic respirometer of oxygen uptake by healthy tissues of full-grown Turkish tobacco leaves, a compound curve is obtained. The results of 3 experiments are illustrated in figure 1. These curves represent the rates of oxygen uptake during progressive suffocation. It is evident that the rate of oxygen uptake by the tissue does not diminish in direct proportion to the lowering of oxygen tension in the surrounding solution. Respiration first proceeds at maximum velocity at the highest tensions of oxygen (first part of the time period), then suddenly (within 2 to 4 minutes) shifts to an intermediate rate, which carries on until one or two more inflections, which result in the lowest rates of oxygen uptake. Further suffocation results in a gradual reduction in oxygen respiration, which, if not relieved by aeration, finally results in death of the tissues. Treatment of leaves with KCN 0.008 molar, which also inhibited the peroxidase and catalase activity of the ground-up tissues as indicated by guiacum and hydrogen peroxide, respectively, reduces the rate of oxygen uptake of the living cells. Polarographic measurements have shown that HCN inhibits those components of oxygen respiration responsible for the high velocities of uptake that occur at the higher oxygen tensions during progressive suffocation (Fig. 2). This is in accord with previous work on *Avena* coleoptiles (8, 14). Analysis of a large number of experiments indicated that at least 3 respiration components by their combined action enable maximum oxygen uptake. Three-step reductions in respiration rate during progressive suffocation were often obtained. Tissue from some leaves, however, failed to give clear-cut 3-step reductions or showed more than 2 inflection points (Fig. 1, curve 3). Measurement of such tissues in solutions saturated with pure oxygen revealed that diffusion of oxygen into the tissues was a limiting factor, for the increase in oxygen tension resulted in a marked increase in the maximal respiration rate. With the polarographic method some limitation in oxygen diffusion to the interior of the tobacco leaf probably always occurs. Such limitations make very difficult any accurate comparisons between healthy

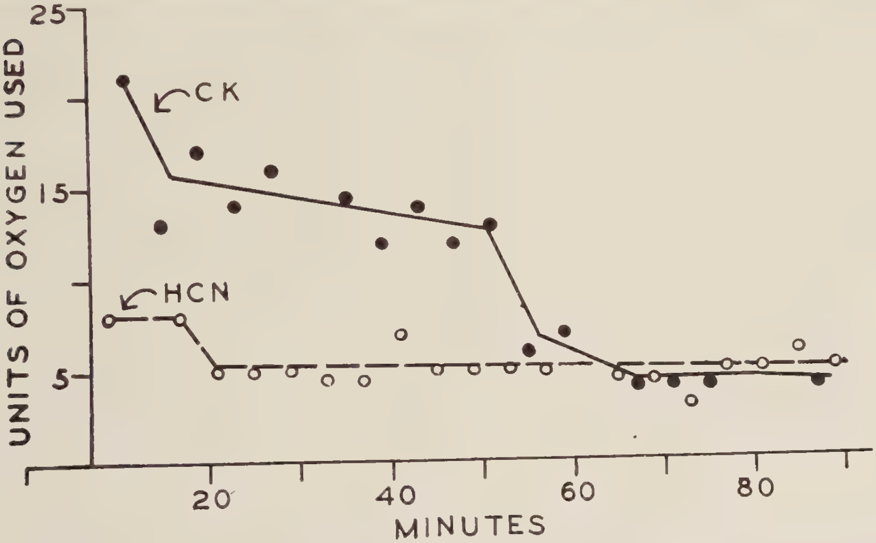

FIG. 2. The effect of HCN on the respiration of healthy Turkish tobacco leaf tissues. Both samples were from the same leaf, oxygen-respiration being measured in the polarographic microrespirometer.

and diseased samples at maximal oxygen consumption in the polarograph. This difficulty is accentuated because of *compensatory* changes that develop in the respiratory catalytic systems that show a differential response to oxygen tensions. As will be pointed out below the multiplication of the virus results in a reduction in the activity of one of the cyanide-sensitive components of respiration that requires a high oxygen tension in order to function, and is followed by a marked increase in a cyanide-resistant component that is able to work at relatively low oxygen tensions. Thus, during progressive suffocation in the polarographic respirometer, the *outer* tissue layers of a diseased sample may exhibit a lower respiration rate than corresponding cells of the healthy leaf, whereas the *interior* cells of the infected sample may respire more rapidly than the corresponding healthy cells. This latter behavior is due to the increased activity in the diseased leaf of the particular respiration component able to function at low tensions of oxygen.

In spite of these limitations the polarographic method proved to be of definite value because the results obtained indicated the existence of different respiration systems in tobacco separable by the techniques of progressive suffocation and enzyme poisoning. It is possible to interpret all of the polarographic data in the light of results obtained from measurements of protoplasmic streaming and of oxygen uptake in the Fenn respirometer.

Measurements of Protoplasmic Streaming. It has been previously determined that protoplasmic streaming in tobacco leaf cells is oxygen-sensitive and responds to cyanide in about the same way that oxygen uptake does (22). A further study was made in the hope that rate of streaming (cyclosis) might be used to more precisely determine the above-mentioned different respiratory components in individual cells. Experiments have shown that the response of tobacco cells to suffocation (diminishing oxygen supply) and cyanide is essentially the same in both cyclosis and oxygen respiration. Three different oxidation components contributing to the rate of protoplasmic streaming have been determined by progressive suffocation (Fig. 3, A and B). The first of these, the ''A component'' ceases to function at a certain oxygen level as measured by cyclosis, later a ''B component'' drops out, whereas at the lowest oxygen tensions protoplasmic streaming is energized only by a ''C component.'' The A and B components also can be reversibly inhibited with NaCN or KCN (Fig. 3, A and B). Potassium cyanide, 0.0002 molar, is as effective as 0.002 molar in reducing the rate of streaming. The relationships between streaming and respiration, apparent from a comparison of figures 1 and 3, are particularly striking when mosaic-diseased and healthy cells are compared (Tables 1 and 2). The action of the virus results in the same characteristic changes in both. Repeated experiments have shown that the rate of protoplasmic streaming can be used as an index to respiratory activity in single cells.

Measurements in the Fenn Respirometer. Measurements of oxygen uptake by healthy leaf tissues in the Fenn respirometer gave results that, in general, accord with those obtained by the polarograph or by protoplasmic streaming determinations. With the Fenn respirometer volumes employed (approximately 7.5 cc.) it was not possible to separate the A, B, and C systems by auto-suffocation. However, respiration inhibitors can be used to advantage. HCN inhibited from 40 to 50 per cent of the total respiration providing certain conditions were realized (Fig. 5). Preliminary investigation demonstrated that *cyanide-sensitive respiration in the interior of the leaf is inhibited in varying degrees unless the stomata in both upper and lower epidermes are kept open,* and excessive mechanical or other injury

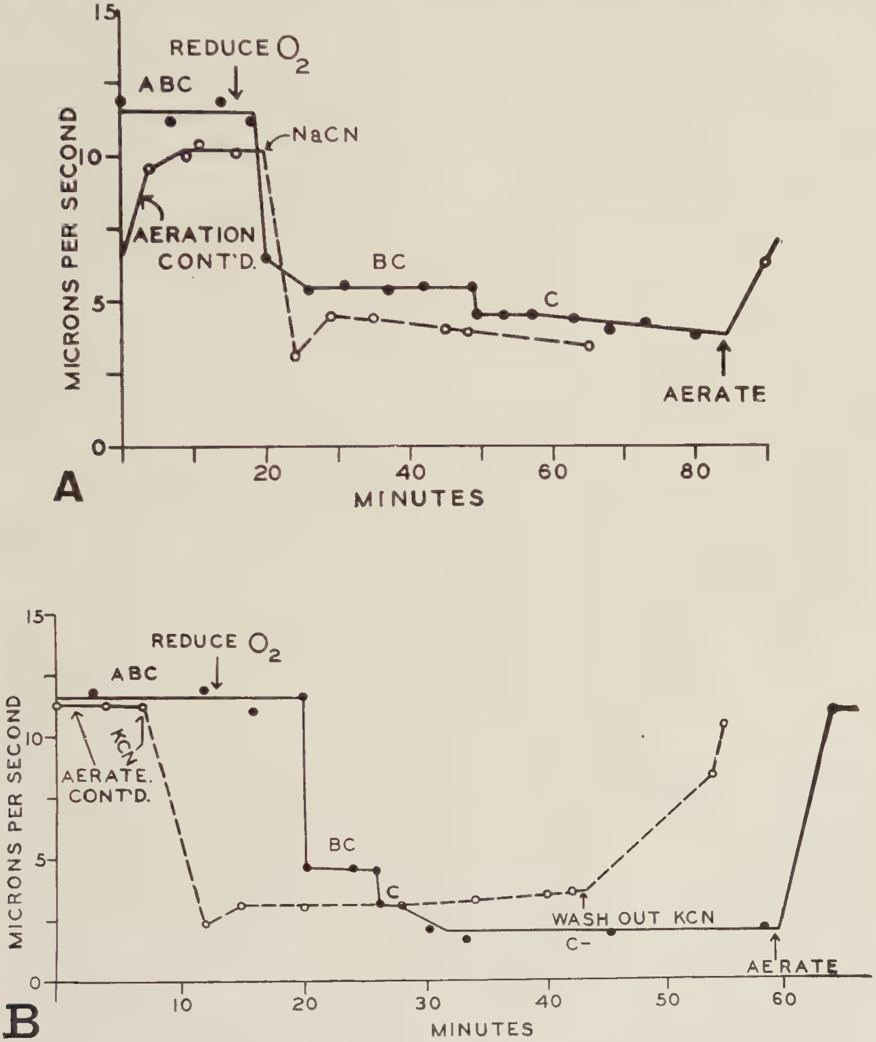

FIG. 3. A. Cyclotic analysis of a healthy epidermal cell of Turkish tobacco. The rates of protoplasmic streaming are recorded in microns per second. Solid line indicates rate during progressive suffocation and the dotted line behavior following treatment with NaCN. The rates of protoplasmic streaming due to the different respiration systems (A, B and C) are indicated on the curve. B. Similar type of analysis of another healthy cell under conditions of more rapid suffocation, and using KCN as the inhibitor.

TABLE 1.—*Activities of oxidation components A, B and C in 8 pairs (healthy-infected) of epidermal cells of Nicotiana tabacum at various periods following inoculation with tobacco mosaic virus (Marmor tabaci). Analyses based on measurements of inflection points during progressive suffocation. Each pair of cells represents typical cells from opposite sides of the same leaf*

Expt. No.	Leaf half in which cell was measured	Hours after inoculation	Relative amount hexagonal crystalline material	Activity of oxidation components A, B and C (rate of cyclosis in microns per second)					Temperature in °C.
				ABC	BC	A	B	C	
1	CK.	....	....	10.9	5.6	5.3	1.5	4.1	27.5
	INOC.	77	*[a]	6.5	5.6	0.9	1.8	3.8	
2	CK.	....	....	13.4	6.9	6.5	1.8	5.1	27.5
	INOC.	80	abnormal vacuolization	12.9	7.6	5.3	2.5	5.1	
3	CK.	....	....	12.6	8.1	4.5	1.9	6.2	28.0
	INOC.	84	*	....	9.3	0	1.5	7.8	
4	CK.	....	... ...	11.5	6.5	5.0	2.3	4.2	27.5
	INOC.	132	**	....	11.3	0	2.2	9.1	
5	CK.	....	....	11.3	7.0	4.3	1.4	5.6	27.5
	INOC.	202	*	....	9.0	0	1.8	7.2	
6	CK.	....	....	13.2	6.5	6.7	2.0	4.5	27.5
	INOC.	215	***	....	8.0	0	1.8	6.2	
7	CK.	....	....	15.0	7.0	8.0	1.7	5.3	28.0
	INOC.	218	***	....	10.4	0	2.1	8.3	
8	CK.	....	....	13.2	6.1	7.1	1.7	4.4	27.5
	INOC.	293	***	....	10.7	0	3.1	7.6	

[a] * = small amount of hexagonal crystalline material; ** = twice as much; *** = three times as much.

TABLE 2.—*Activity of cyanide-insensitive oxygen respiration in leaves of N. tabacum after inoculation with tobacco mosaic virus (all measurements made in Fenn respirometer)*

No. of leaf	Hours after inoculation	Micro liters O_2 consumed per hour (samples approx. 120 mg.)		Percent. increase in O_2 uptake in inoculated half	Test for virus in expt. samples (uninoc./inoc.)[b]	Temp. during test in °C.
		Uninoculated half	Inoculated half			
1.	not inocul.	120.8 (left)	126.0 (right)	dif. = 4.3	....	27.5
2.	Do	40.6 (left)	40.8 (right)	dif. = 0.5	....	27.4
3.	30	53.4	51.5	− 5.5	0/39[c]	27.5
4.[a]	51	62.0	60.6	− 2.3	0/105[c]	27.7
5.	92	50.2	50.2	0.0	31/634[c]	27.6
6.	116	62.0	81.6	24.1	5/670[c]	27.6
7.	123	56.8	67.0	15.3	0/844[c]	27.7
8.	168	56.9	80.6	29.5	0/648	27.5
9.	198	92.1	117.7	21.8	9/778	27.8
10.	216	25.6	46.6	45.1	0/1915	27.5
11.	218	41.5	49.2	15.7	3/1043	27.7
12.	237	15.3	30.8	50.4	0/264	28.4
13.	291	66.5	76.8	13.5	11/649	27.8

[a] This sample run with closed stomata only, no HCN was used.

[b] Number of local lesions developed on half leaves of *N. glutinosa* hybrid.

[c] Uninoculated and inoculated samples tested on different plants of the *N. glutinosa* hybrid.

TABLE 3.—*Rates of cyclosis in epidermal cells extending from the yellow margin of a primary lesion of yellow mosaic into the normal-appearing area around the periphery. Cell position number one was nearest to the center of the lesion*

Position	Mosaic-diseased cells Rate of cyclosis in microns per second		Healthy cells Rate of cyclosis in microns per second	
	Before KCN	In KCN[a]	Before KCN	After KCN[a]
1	10.7	11.1	11.6	4.4
2	10.2	11.4	11.3	4.3
3	11.1	8.7	10.7	4.1
4	8.4	7.3	9.5	4.0
5	8.4	6.5	8.9	3.9
6	8.1	5.2	....	
7	Not measured	6.3	......	......

[a] Cells 1 and 2 are not the same cells as 1 and 2 in the first column, although they were in the same relative position with respect to the lesion. Cyanide actually caused a decrease in cyclosis, although much less inhibition occurred in the diseased cells.

avoided. When leaf samples were removed about 6 p.m. and floated on water in covered Petri dishes, about 40 cm. from a 100 watt Mazda lamp, the stomata were wide open by the next morning. Samples so treated could be held in the dark for periods of ½ to 2 hours without the stomata closing. Leaves treated in this way were the only ones that gave both maximal respiration and response to cyanide.

As a result of studying oxygen respiration with the 3 techniques described above it can be concluded that oxygen uptake in healthy Turkish tobacco leaves proceeds through the combined action of at least three systems. The first of these, the "A system" is very labile, being readily blocked by cyanide or by reduction in oxygen tension. The "B system"

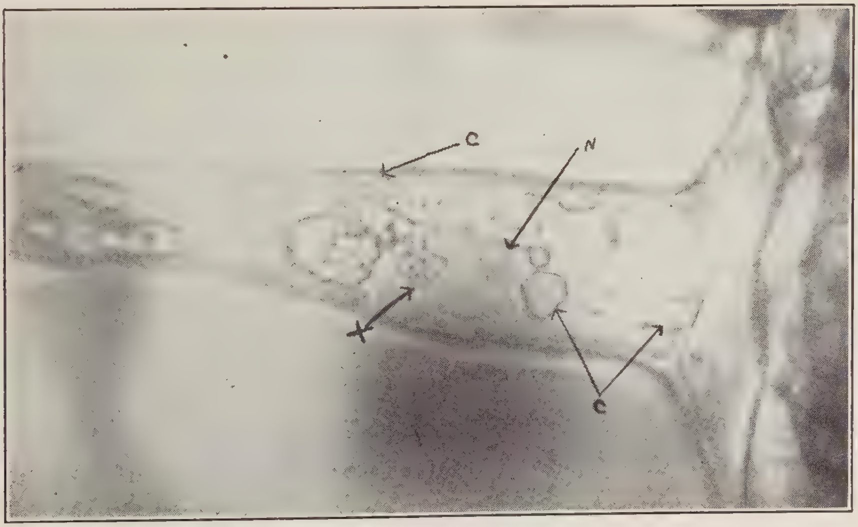

FIG. 4. Living trichome from leaf margin of *Nicotiana tabacum* Turkish infected with the "green" strain of *Marmor tabaci* Holmes. Hexagonal protein crystals, C; nucleus, N; intracellular body ("X body"), X. Approx. ×2,000.

is less labile, but can be blocked by cyanide or further reduction in oxygen tension. The third, or "C system" is the most resistant to suffocation and is not directly blocked by cyanide. Furthermore, in young leaves, the cyanide-sensitive systems are more active than in older leaves, sometimes constituting over 80 per cent of the total respiration.

The Effect of Tobacco Mosaic Virus on the A, B, and C Systems

The first respiratory symptom of infection with *Marmor tabaci* is a reduction in activity of the A system (Table 1). This effect can be detected

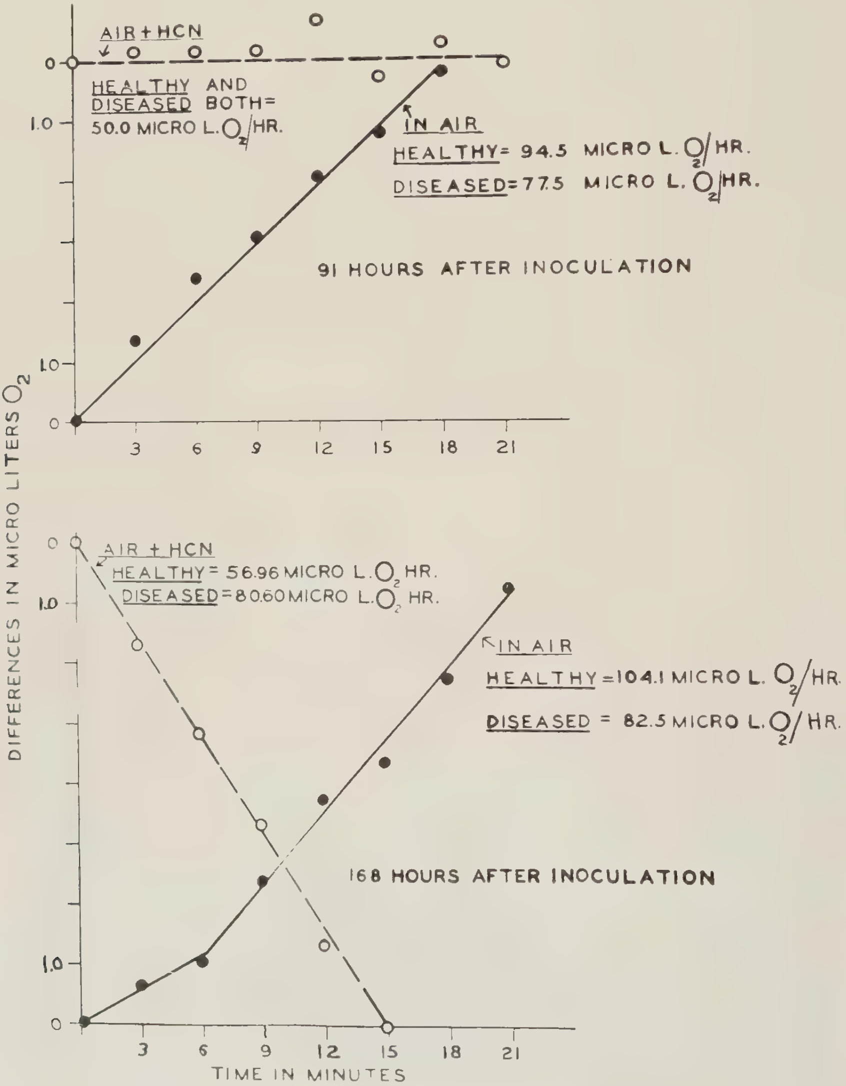

FIG. 5. Oxygen uptake in mosaic-diseased and healthy Turkish tobacco leaf tissue, 91 and 168 hours after inoculation. Experimental samples each consisted of 6 square centimeters (approximately 120 mg.) of tissue. Differences in oxygen uptake between the diseased and healthy samples are plotted. The absolute rate of oxygen uptake in micro liters per hour is indicated in the figure.

by cyclotic measurements in individual cells 72 hours, or less, after inoculation. It becomes manifest just before or shortly after the development of the first intracellular hexagonal protein crystals. These crystals are positive evidence of cellular infection by the virus (Fig. 4). For a detailed description of the cytological changes following mosaic infection, see Dufrenoy and Dufrenoy (10). The C system is not changed in activity until the A component is inactivated. Measurements in the Fenn respirometer have not given positive evidence of reduction in activity of the A system before 91 hours after inoculation (Fig. 5). Figure 5 shows a 53 per cent reduction in oxygen uptake in the presence of cyanide 91 hours after inoculation without apparent change in the cyanide-resistant system. Measurements made with the cyclosis technic in a number of cells from leaf margins 48 to 72 hours after inoculation indicate that the reduction of respiration in individual cells may occur within 48 hours or less. It is believed, however, that inactivation of the A system occurs only after development of large quantities of virus. This is borne out by the relationship exhibited to formation of the hexagonal crystals, which probably indicate a high virus titre (2).

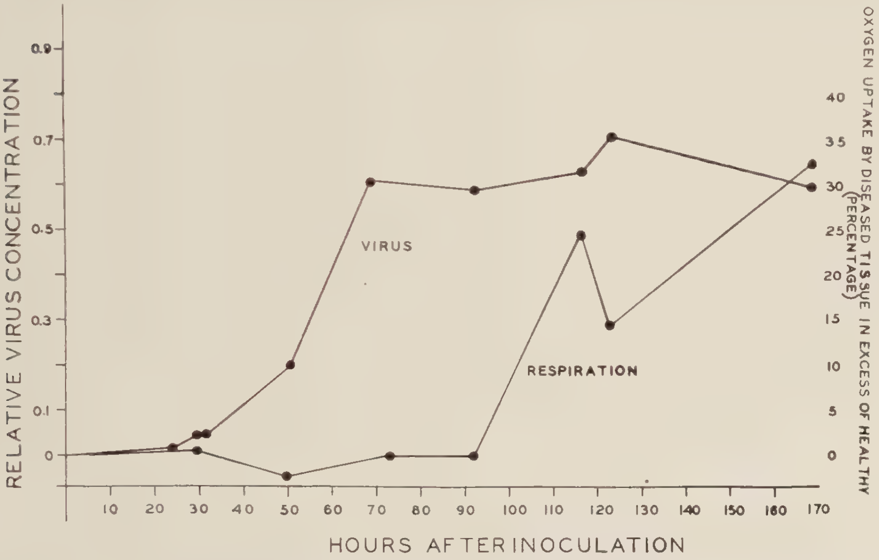

Fig. 6. The effect of *Marmor tabaci* Holmes on cyanide-resistant oxygen respiration in *Nicotiana tabacum* Turkish. The relative virus concentration has been plotted against the cyanide-resistant respiration in the same samples. Respiration is expressed as oxygen uptake by the diseased tissue in excess of the healthy tissue of the same leaf.

The activity of the B and C systems following infection are illustrated in table 1. It can readily be seen that 84 hours after inoculation there is a large increase in the activity of the C system. Apparently, there is little or no significant change in the activity of the B system. The relation between the approximate curve of virus multiplication and increase in activity of the C system as measured with the Fenn volumeter is shown in figure 6. It is evident that the change in the C system does not occur until after virus multiplication has practically stopped. The increases in oxygen uptake by the C system are easily demonstrated in the Fenn respirometer by making determinations in the presence of HCN (Table 2, Fig. 6). These increases in oxygen uptake by the C system parallel increases in activity of the C system as measured by protoplasmic streaming (Table 1).

The progressive increase in activity of the C system can also be demonstrated by measuring, cell by cell, the rate of protoplasmic streaming from the center of a lesion to the normal appearing peripheral areas (Table 3). In this case the epidermal cells over the yellow lesioned area were extremely resistant to cyanide, whereas the more recently infected cells, further from the periphery of the visibly affected area, behaved more like normal cells.

In another test involving the ''yellow strain'' of the virus, the average rate of cyclosis in 5 infected cells was 8.5 μ per second. Following treatment with KCN the average rate was 5.7 μ. In 5 healthy cells of the same leaf the respective values were 10.8 and 3.8 μ per second. In a third test, 197 hours after inoculation, 5 infected cells exhibited an average cyclotic rate of 9.2 μ per second and 5.4 μ per second following treatment with cyanide. Corresponding data for 5 healthy cells of the same leaf were 10.7 μ per second and 2.4 μ per second, respectively. The general effects of the ''yellow strain'' on cellular respiration are similar to those produced by the parent ''green'' virus.

The first macroscopic symptoms of infection (chlorophyll destruction) with either the ''green'' or ''yellow'' strain of the virus usually appeared in the inoculated leaves 48 to 96 hours after inoculation. The ''green strain'' of *Marmor tabaci* produced very faint yellowish-green primary lesions in inoculated leaves, whereas the ''yellow strain'' produced very definite yellow local lesions. An increase in C system activity does not appear to be a necessary concomitant of chlorophyll destruction, because the latter is sometimes apparent before any change in the C system activity can be observed. In the early stages of infection with either virus cyclosis tends to become jerky and irregular, later stages concomitant with increases in C system activity are characterized by remarkable regularity and smoothness of protoplasmic streaming, together with the disappearance of larger cytoplasmic granules.

In some leaves cyclosis in both healthy and diseased cells shows a secondary poisoning effect with cyanide that is entirely distinct from ordinary reversible partial inhibition of protoplasmic streaming. When present this auto-inhibiting effect in the presence of cyanide is much more marked in diseased than in healthy cells. In these peculiar cases treatment with cyanide for 30 to 60 minutes results in accumulation of some substance or substances highly toxic to the C system. This phenomenon, which is of only occasional occurrence, will be the subject of a later paper.

DISCUSSION

Aerobic respiration of tobacco leaves involves both cyanide-sensitive and cyanide-resistant systems of catalysts. Similar systems have been demonstrated for the respiration of other phanerograms (3, 5, 6, 8, 13). While it has not been proved, cyanide-sensitive respiration of tobacco leaves (A and B components) probably is attributable at least in part, to the activity of a cytochrome oxidase-cytochrome system. At present it is not possible to state the fundamental difference between the A and the B components, although they can be separated by varying the oxygen tension. During respiration at low oxygen tensions, or in the presence of cyanide, molecular oxygen may enter the process through the re-oxidation of the alloxazine nucleotide which is a cyanide insensitive respiratory enzyme (15, 16, 17). Thus our C system would correspond to the complex of dehydrogenations and hydrogen transfers extending from the substrate to the final donation of hydrogen by the reduced alloxazine nucleotide to oxygen. In the absence of cyanide, or, at an adequate oxygen tension, the cytochrome system with the corresponding dehydrogenases would bring about the re-oxidation of the alloxazine nucleotide, and oxygen would be "activated" by cytochrome oxidase, thus accelerating the acceptance of hydrogen. Under these conditions a more rapid uptake of oxygen would occur and the greater supply of energy would accelerate protoplasmic streaming proportionally (9). While this may represent a partial definition of the cyanide-sensitive and cyanide-resistant respiration systems in tobacco, experimental data on the exact chemical nature of higher plant respiration are too scant to warrant detailed conclusions. It is important, however, that certain respiratory systems in tobacco can be differentiated by the techniques of progressive suffocation or cyanide treatment. DuBuy and Olson (8) have described similar systems in *Avena* coleoptile using the polarographic respirometer and measurement of protoplasmic streaming. Marsh and Goddard (13) found, in mature leaves of carrot, only cyanide-resistant respiration and both cyanide-resistant and cyanide-sensitive respiration in young leaves. In *Avena* (8), however, the cyanide-sensitive system is always active, although it is greatly reduced in older plants. A similar situation occurs in tobacco.

It has been variously reported that viruses stimulate, inhibit, or have no effect on the respiration of plants susceptible to them. Cordingley, *et al.* (7) have observed that mosaic-infected tobacco leaves resemble *old* healthy leaves in their carbohydrate and protein metabolism. These authors suggested a possible lower respiration rate in mosaic-diseased leaves from their data for loss of carbohydrate.

Caldwell (4) reported increased CO_2 output by leaves of tomato infected with aucuba mosaic. This was true in normal atmosphere or in oxygen. Caldwell and Meiklejohn, using thin slices of tomato stems in Barcroft respirometers (5, 6), found that inhibition in respiration produced by M/300 cyanide was of the same order in aucuba-mosaic-diseased and healthy tissues. However, it is suggested (5, p. 485) that ". . . the enzymes concerned in the preliminary stages of respiration were more active in virus-diseased than in healthy plants." Dunlap (11) observed that leaves of young tobacco plants infected with mosaic evolved more CO_2 than similar healthy leaves. The reverse situation held in mature leaves. Dunlap worked with field-grown plants naturally infected with mosaic. Dufrenoy and Dufrenoy (10), in a physiological and cytological study of the tobacco-mosaic disease, found greater oxygen uptake in infected cells of old tobacco leaves than in apparently healthy cells; whereas, in bud tissues, there was a reduction in oxygen uptake of over 30 per cent following infection. These results can be harmonized with the data obtained in the present investigation on the assumption that in infected buds the virus brings on an inhibition of cyanide-sensitive respiration ("A system") without at first causing an elevation in the cyanide-resistant respiration ("C system"). The increase in respiration occurring in old leaves could be accounted for on the basis of secondary increases in the relatively stable cyanide-resistant "C system."

It is not possible to explain the variance between some of these reports and the present investigation, although differences in methods of sampling or in other techniques may be involved. The use of the half-leaf technique in conjunction with different micro methods for determining respiratory activity has eliminated many variables that in certain cases make interpretation difficult. In fact, the protoplasmic-streaming technique allows measurement of respiratory activity in *single* cells. Moreover, it is a convenient and rapid method of assaying respiratory conditions in a tissue.

The primary respiratory symptom of infection with tobacco-mosaic virus is one of inhibition. A secondary symptom is a stimulation of the cyanide-resistant portion of the respiratory mechanism. If in other investigations the metabolism of a healthy plant were compared with that of diseased plants in late stages of infection, and if no special precautions were taken to insure open stomata, it is possible that the oxygen tensions within the tissues were inadequate to allow full activity of all respiratory systems. Thus the sum total of respiration of the healthy tissue could consist of a small part of the A system activity plus the B and C components. In the diseased tissues it would consist of the activity of the B system plus that of an increased C system. This would give an apparent increase in respiration in the diseased tissues. While the virus affects the respiration systems of the suscept cell, these systems in their turn apparently affect the multiplication of the virus, as shown by our data. This multiplication apparently depends upon a cyanide-poisonable component of the cell (21). Recent work with gaseous cyanide (Woods, unpublished) has further demonstrated the specific and *reversible* nature of the inhibition of virus multiplication. The virus apparently reaches a high concentration before the "A system" of respiration ceases activity. Since the "B system," which is also cyanide-sensitive, seems to be little affected by the virus and continues to function beyond the cessation of virus multiplication, it is possible that the A component, or something connected directly with it, is necessary for virus multiplication. If the A system ultimately is inhibited or destroyed as a result of activity of the virus, the latter might thus bring about a condition preventing its own further increase.

A connection between certain oxidizing enzymes and virus multiplication already has been indicated by Woods (18, 19, 20) who has called attention to the relation of cellular oxidases and peroxidases to virus diseases of plants. While it is unlikely that the virus protein is, itself, of the nature of a peroxidase, there probably are fundamental relationships between multiplication of this protein and specific oxidizing mechanisms of the cell, as Woods indicated a number of years ago.

SUMMARY

Oxygen respiration in healthy Turkish tobacco leaves is accomplished by both cyanide-sensitive and cyanide-resistant systems of catalysts. The energy for protoplasmic streaming is derived from these respiration catalysts. Rate of streaming can be used as an index to cellular respiration.

There are two cyanide-sensitive systems, separable from each other by means of their "critical oxygen tensions"; these have been arbitrarily designated as the A and B systems. The third or "C system" is resistant to cyanide and continues to function at lower oxygen tensions than either the A or B components during progressive suffocation.

Infection with tobacco mosaic virus (*Marmor tabaci* H.) results in an inhibition in the A system 48 to 72 hours after infection of the cell. The B system apparently is not affected, but the C component is greatly increased in activity following inhibition of the A component.

The inhibition of the A system apparently occurs only after development of a high concentration of virus, and such inhibition of certain respiratory components may be the factor preventing further multiplication of the virus protein.

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A BACTERIAL NECROSIS OF THE GIANT CACTUS

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INTRODUCTION

Bacterial necrosis of the giant cactus (*Carnegiea gigantea* Britt. and Rose (*Cereus giganteus* Engelm.)), long present in southern Arizona, is a serious, hitherto undescribed[1] disease. Its recent encroachment on cactus parks and privately owned estates has made necessary the investigational work described in this paper.

IMPORTANCE

Bacterial necrosis has been observed in an area 200 miles broad, north and south, by 250 miles long, east and west. The disease is particularly destructive near Tucson, in Picacho Park, and near Maricopa; it occurs between Sacaton and Chandler, northwest of Phoenix, westward to Yuma, and along the Ajo highway from Tucson to the Mexican border. The malady varies in severity in different forests of giant cactus. For example, plants killed by necrosis plus living cacti with visible infection, on five 1-acre tracts in the Saguaro National Monument, amounted to 21, 0, 0, 4, and 18 per cent, respectively. The percentages on 3 similar tracts in the Santa Catalina foothills were 33, 13, and 9, respectively.

The figures on mortality and infection become serious when the comparatively rapid disappearance of the fallen fleshy cacti is considered together with the probability that many plants in the earlier stages of infection are missed in field reconnaissance. Most deaths counted probably occurred within the past 2 years, and many cacti in the forests studied are certainly condemned to death within a short time. Few seedlings are coming on to replace the dead and dying plants. It appears possible that bacterial necrosis may explain some growths of giant cactus in southern Arizona that are apparently on the way out (Fig. 1, *B*).

Bacterial necrosis is important economically, as well as botanically. The cactus that it destroys is an attractive feature for the many tourists that annually visit Arizona, and financial income from such visitors is greater than the income from many other sources. The disease has already invaded the estates of wealthy citizens who spend the winter in southern Arizona and whose requests for information on the necrosis have stimulated our studies.

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[1] A brief note by Dr. Forrest Shreve of the Carnegie Desert Laboratory may have reference to bacterial necrosis: "When struck by lightning or wounded in any other manner during the dry season, it (the giant cactus) recovers very rapidly by the formation of a heavy callus over the wounded spot. If it is wounded in the rainy season, however, bacterial decay sets in very rapidly and a large plant may be destroyed in less than a week as a result of a small wound."—From Britton and Rose, The Cactaceae 2: 166. 1920.

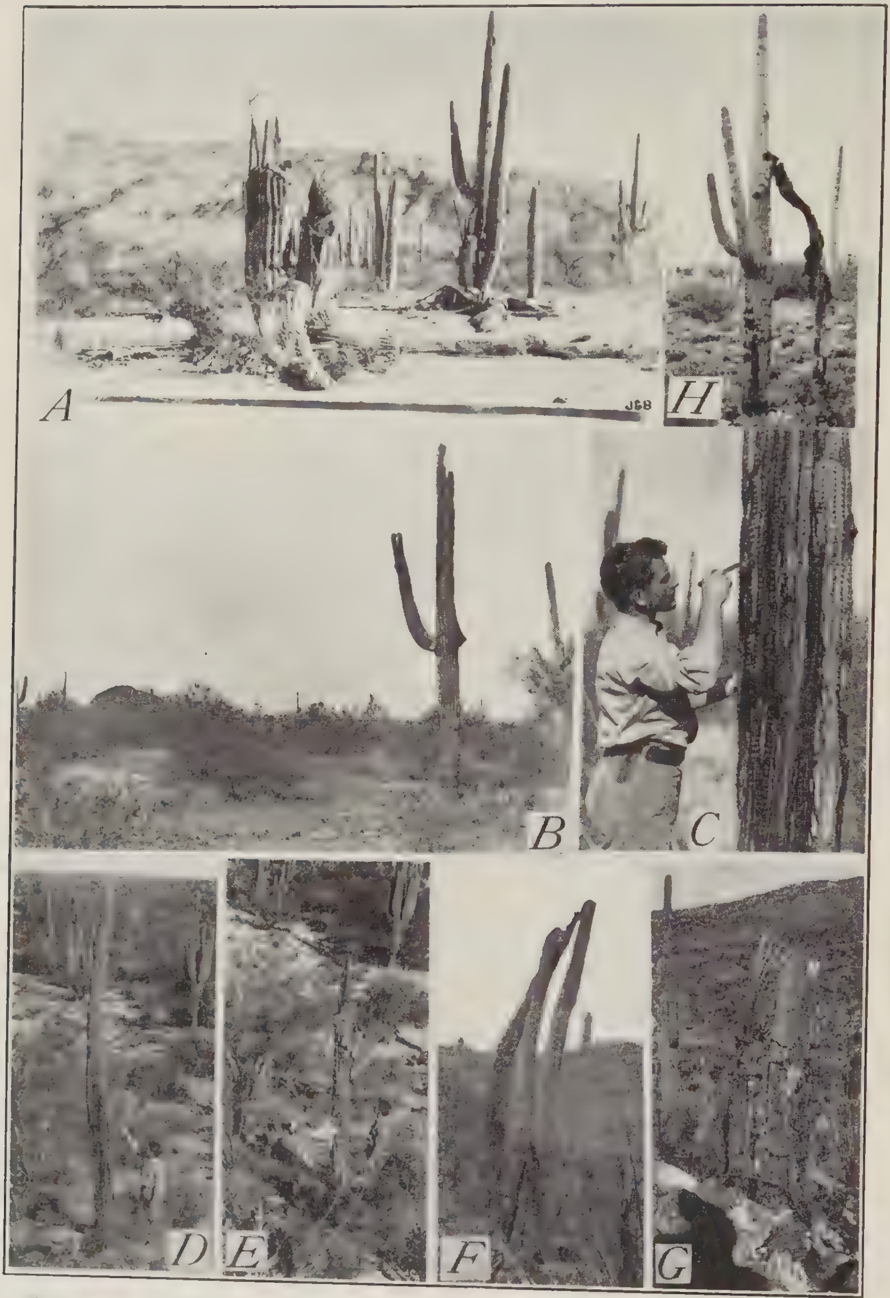

FIG. 1. *A*. Destruction of giant cactus, *Carnegiea gigantea*, resulting from bacterial necrosis. *B*. Thin growth of giant cactus believed to be mainly due to necrosis. Cactus in right foreground is "bleeding" at top and base. *C*. Close-up of part of trunk of infected cactus with streams of bacterial ooze; new stream below knife point. *D, E*. Rate of progress of necrosis: *D*, giant photographed February 10, 1940, and *E*, same plant November 2, 1940. *F*. Spread of disease in group: one cactus broken, a second is leaning against another plant; the two remaining cacti are badly infected. Taken February 10, 1940. *G*. Group of cacti illustrated in preceding photograph. Taken November 2, 1940. *H*. Spread of disease from one individual cactus to another. The decayed cactus fell against a branch of an adjacent plant to which it carried the necrosis.

SYMPTOMS

The first symptom of the bacterial necrosis of *Carnegiea* is a small, circular, light-colored spot, usually with water-soaked margin, on the surface of the integument of trunk and branches. Underneath the surface discoloration the soft parenchymatous tissues (Fig. 2, *A* and *B*) become water-soaked after bacterial invasion and subsequently brown to almost black. As the infection progresses the spot enlarges and assumes a purplish hue. In cases in which the infection advances rapidly the central part of the spot breaks (Fig. 1, *A* and *B*) and a brown liquid exudes. Rapid destruction of the tissues is then accompanied by "bleeding" (Fig. 1, *B*, *C*, and *H*) but slower internal decay may proceed without exudation. The rotted tissues dry, break up into granular to lumpy pieces and fall to the ground, leaving the woody stelar strands bare (Fig. 1, *A* and *G*).

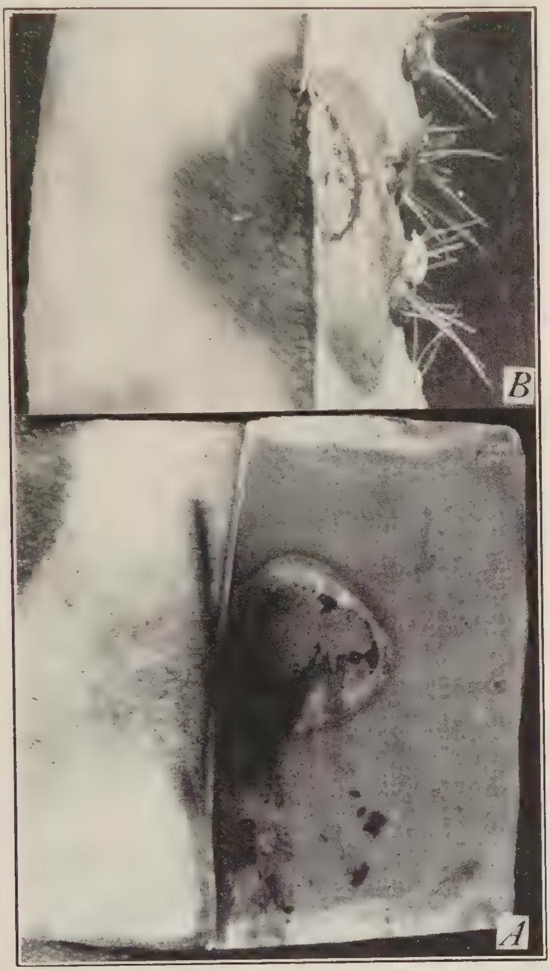

FIG. 2. Necrotic spots, *A*, natural, and *B*, artificial. Note discolored broken surface at right and darkened internal tissues, left. Elongated vertical streak in *A* is an artificial cut.

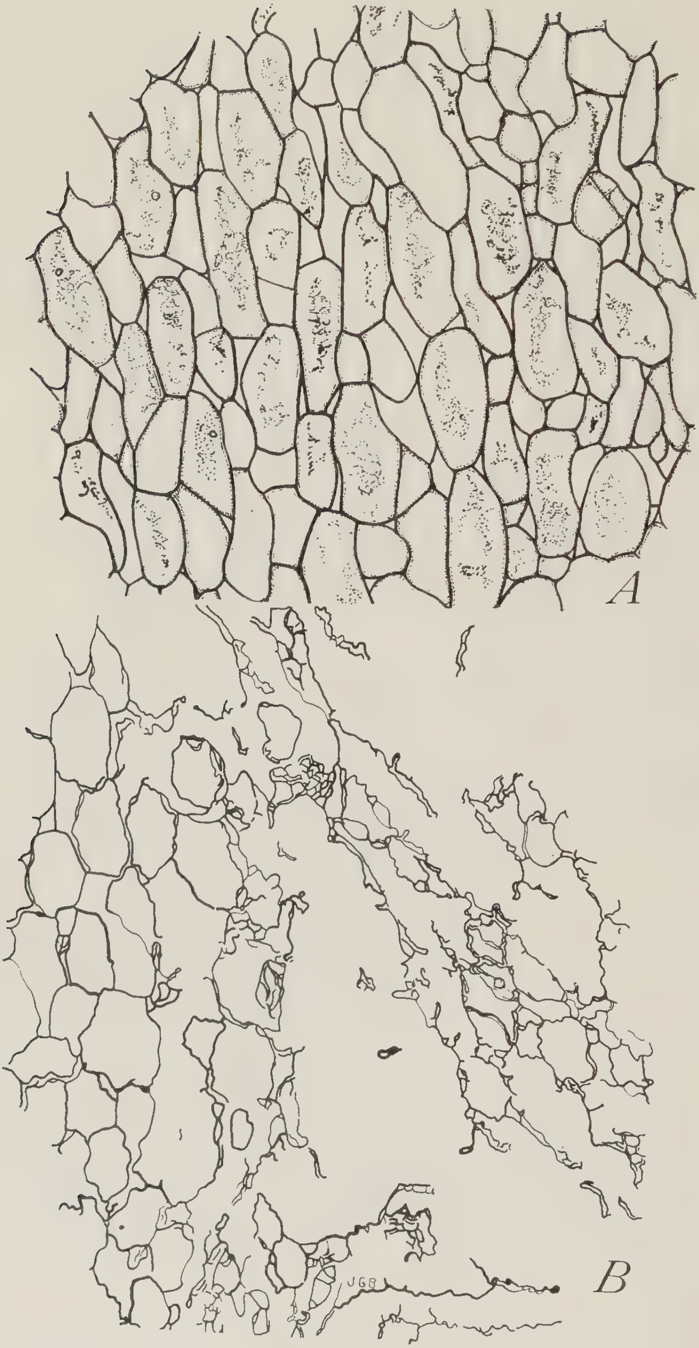

FIG. 3. *A*. Part of transverse section of healthy parenchyma consisting of turgid cells. *B*. Comparable section of necrotic tissues partly broken down with cavities. × 52.

Necrosis may start anywhere on the trunk and branches. Where it girdles the columnar trunk near the base, the plant is so weakened that the latter easily breaks in a windstorm. Such broken giants are only too common in the forests studied (Fig. 1, *A*). Decay at the top of the trunk leaves the woody ribs of the stele there exposed. Rotting on one side is followed by the leaning of the trunk toward that side (Fig. 1, *F*). Girdling of large branches at their bases causes the branch to sag and break off. Eventually the giant falls or, if it remains erect, the infected parenchyma disintegrates, exposing the bare woody skeleton. The necrosis kills quickly, often within 2 or 3 weeks, and in the course of a few months the affected tissues are almost completely broken down (Fig. 1, *E* and *G*).

HISTOLOGIC AND CYTOLOGIC CHANGES

Direct histologic and cytologic changes resulting from infection are limited to the integument (cuticle-covered epidermis and thick-walled hypodermal cells) and the underlying parenchymatous tissues, both within and without the stele. The drying, cracking, and discoloration of the cuticle-covered epidermis and the hypoderma have already been mentioned. The chlorenchyma loses its chlorophyll, becomes water-soaked and discolored brown to black; all other parenchymatous tissues likewise show water-soaking and discoloration. Finally, the soft tissues break down (Fig. 3, *A* and *B*) into a dark colored, almost odorless liquid unless slow destruction affords time for drying.

Cytologic changes affect the entire parenchyma cell. The wall loses its middle lamella by dissolution of the latter (Fig. 4, *H, I,* and *J*), so that the cells in transection appear to have a double wall separated by a narrow, more or less irregular space instead of a single, continuous wall. In other cases the unequal dissolving of the middle lamella leaves numerous cavities (Fig. 4, *I*) in the lamellar region. The remainder of the wall eventually becomes soft and irregular in outline (Fig. 4, *D, F,* and *J*). The disintegrating wall is not uniform in consistency, as shown by more persistent fibrous streaks (Fig. 5, *A* to *D*) in its mass. Quite early in the infected tissues nuclear changes are evident; the nuclear membrane becomes thickened (Fig. 4, *D* and *E*); the nucleoplasm loses its reticular aspect (Fig. 4, *C* to *G*) and becomes more or less homogeneous and retentive of stains. The nuclear mass may be considerably increased in bulk and the nuclear outline irregular and wrinkled; shrinking and disintegration follow. The nuclei of chlorenchyma cells become surrounded with chloroplasts (Fig. 4, E) that, like the former, assume an increased avidity for certain stains. The chloroplasts finally shrink and disappear.

The node of disintegration of the cell wall in bacterial necrosis deserves special emphasis in view of recent microchemical investigations (1) in which the walls of plant cells have been observed to consist of fibrils held together by a pectic substance. The fibrils are microscopic elongated particles of cellulose joined end to end. This fibrillar structure is evident in the disintegrating cell walls in the giant cactus. Swelling of the pectic matrix (Fig. 5, *B* and *C*) separates and somewhat disarranges the fibrils. Dissolution of the middle lamella is usually first noticed, followed by the rapid breakdown of the matrix and less rapid dissolution of the fibrils.

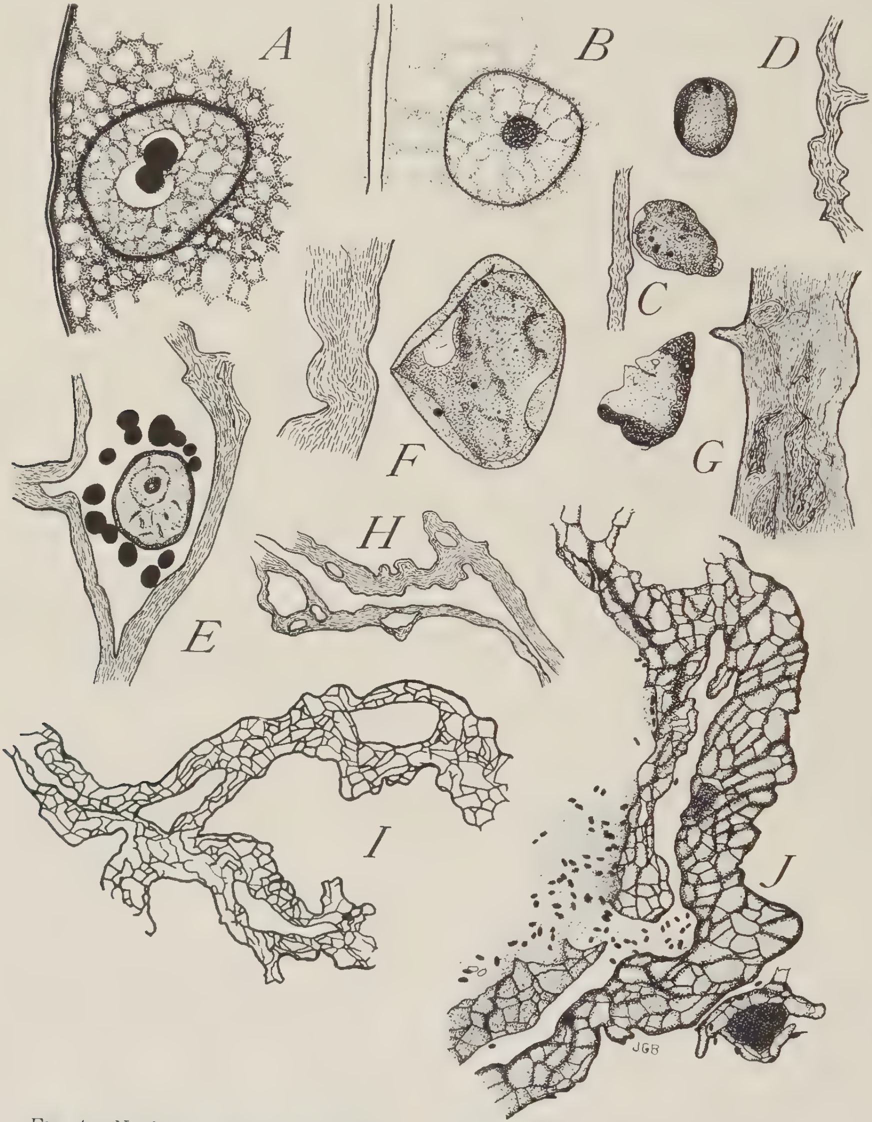

FIG. 4. Nuclear and cell wall studies. *A* and *B*. Normal nuclei and adjacent parts of cell wall. *C*. Nuclear reticulum lost; nucleus shrunken; wall somewhat swollen. *D*. Nuclear membrane thickened; wall considerably swollen and softened. *E*. Nucleus of chlorophyll cell with thickened membrane and with chloroplasts clustered around it. *F*. Disintegrating nucleus and much swollen cell wall. *G*. Later stage in disintegration of nucleus and in swelling of cell wall. *H*. Part of wall with middle lamella dissolved and cavities in outer layers of the wall. *I*. Later stage in dissolution of cell wall. *J*. Part of swollen cell wall with bacterial action in progress. All drawings made with camera lucida, ×1159.

Eventually, only a little of the wall remains (Fig. 5, *D*). Although like destruction of the walls of plants by a saprophytic soil bacterium has been described (2), similar stages in the disintegration of the walls of living plants through the action of parasitic bacteria appear to have been over-

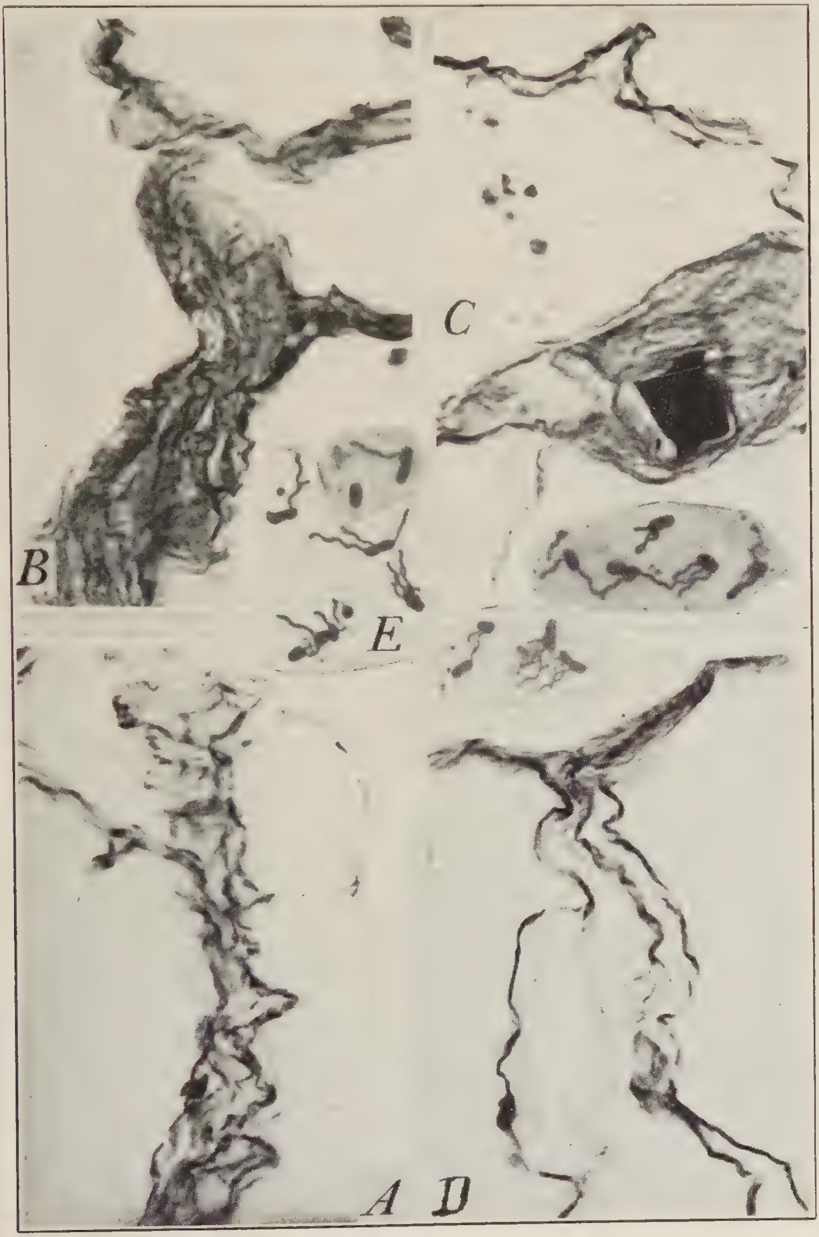

FIG. 5. Photomicrographs. *A*. Piece of swollen wall showing cellulose fibrils and pectic matrix. *B*. Fibrils separated by swelling of matrix. *C*. Later stage in swelling of cell wall, in center; wall above and below is almost gone. *D*. Last stage in decomposition of wall. *E*. Groups of the bacterium of necrosis. *A, B, C, D,* approximately × 1200.

looked by plant pathologists. They should be found in studies of the vegetable rots.

BACTERIOLOGICAL TECHNIQUE

Cubes of the diseased tissues of the giant cactus approximately 5 mm. on a side were cut out aseptically and either placed directly on agar slants or immersed in mercuric chloride solution (1:1000) for 1 min. before culture on agar. Some pieces of the tissues were incubated at room temperatures (22–37° C.) and other pieces at a constant temperature of 26° C. The pieces sometimes gave bacterial growth in 15 to 24 hr. but usually only after 3 or 4 days. Although stained samples of the colonies directly obtained from infected tissues often showed only one morphological form of bacterium, segregation by Koch's method (5) and by streak culture (4) was employed. From the resulting colonies of bacteria transfers were immediately made to potato-dextrose yeast-infusion agar and, from the latter, dilution plates were again poured. The pure cultures of bacteria thus obtained were identical, one culture with another, and also with pure cultures usually given by carefully surface-sterilized blocks of infected cactus tissues.

The bacterium from the necrotic tissues was cultured on 15 different kinds of solutions used as sources of carbon. For this work Dunham's tubes were used, each tube containing 10 cc. of nutrient broth, 0.5 per cent of the desired, separately sterilized carbon source, and 0.5 cc. of 0.4 per cent phenol-red solution. In addition, media consisting of 1 per cent and 2 per cent peptone-dextrose and 2 per cent peptone-levulose, respectively, in distilled water, were seeded with the bacterium. Other media commonly used in bacteriological laboratories, such as litmus milk, gelatin, lead acetate agar, plain nutrient broth, Uschinsky's solution, and malt-extract broth were employed in testing the bacterium from the necrotic giant cactus.

CHARACTERISTICS OF THE BACTERIUM

The bacterium (Fig. 5, *E*) that causes necrosis of the giant cactus is a greyish-white, actively motile, (15–24 hr., agar slants) peritrichiate (8), Gram positive (6), nonsporiferous, very slowly gelatin-liquefying (20° C.), nitrate-reducing, non-milk-curdling, aerobic short rod with rounded ends, occurring singly or in pairs, growing on the surface of agar poured plates in the form of round, slightly raised, smooth, grey-white wet-shining colonies having an entire, well-defined margin. Nutrient broth gave abundant growth. In Uschinsky's solution growth was moderate, liquid turbid, a slight ring appeared at the surface, and sediment was viscid upon agitation. Litmus milk was slightly pink or reduced with no curdling. There was no noticeable odor in any of the media. Acid and gas with abundant growth were produced with the following sources of carbon: arabinose, dextrose, galactose, levulose, maltose, sucrose, raffinose, mannitol, and salicin. For the bacterium is proposed the name **Erwinia carnegieana** Standring n. sp.

INOCULATIONS

Needle inoculations with **Erwinia carnegieana** n. sp. from numerous cultures, hypodermically made into trunk and branches of the giant cactus gave a straw-colored surface discoloration, like that observed in the field, in 3 days; in 5 days the spots were reddish-purple around the needle puncture, with a band of pale-yellow beyond; in 11 days the spreading spots had a water-soaked margin. Internally, the parenchyma (Fig. 2, *B*) was water-soaked and brown to black, again like the change observed in nature. Sections of living and of killed, stained tissues from artificially inoculated giant cactus presented the same pathologic picture of histologic and cytologic changes previously described in this paper for the tissues of plants naturally infected in the field. From the artificially inoculated tissues was recovered the bacterium originally isolated from naturally infected tissues of the giant cactus and inoculated into the healthy plant.

LITERATURE REVIEW

No reference to a bacterial necrosis of the giant cactus, excepting the previously cited note, has been found in literature. At least two bacterial

TABLE 1.—*Comparative characteristics of B. cacticidus, B. cactivorum, and E. carnegieana*

Character	*Bacillus cacticidus*		*Bacterium cactivorum*	*Erwinia carnegieana*
	Liquid	Solid	Liquid and solid	Liquid and solid
Shape	Rods	Coccoid	Rods	Rods
Size	1.3 μ × 0.8 μ	0.8 μ in diameter	1.5 μ × 0.8 μ	1.12 μ–1.79 μ × 1.56 μ–2.90 μ
Grouping	Singly or in pairs		Not in chains	Singly or in pairs
Motility	Active		"Mobile"	Active (flagellate)
Gram's stain	Negative		Positive	Positive
Color	Dirty white to yellow		Not reported	White
Capsule	None		Not reported	Present
Spores	None		None	None

TABLE 2.—*Reactions*[a] *of B. cacticidus (4), B. cactivorum (7), and E. carnegieana n. sp.*

Media	*Bacillus cacticidus*			*Erwinia carnegieana*			Media	*Bacterium cactivorum*			*Erwinia carnegieana*		
	Acid	Growth	Gas	Acid	Growth	Gas		Acid	Growth	Gas	Acid	Growth	Gas
Arabinose	0	...	...	x	4	3							
Dextrose	x	...	...	...	...	...	Peptone-dextrose, 1%	...	0	...	...	4	4
Galactose	...	...	...	x	4	3							
Levulose	...	...	...	x	4	3	Peptone-dextrose, 2%	...	0	...	...	4	3
Lactose	0	...	...	0	3	0							
Maltose	0	...	...	x	4	4	Peptone-levulose, 1%	...	0	...	...	4	4
Sucrose	x	...	...	x	4	4							
Dextrine	...	...	...	...	...	...	Peptone-levulose, 2%	...	0	...	...	4	3
Inulin	...	...	...	0	3	0	Malt broth	x	3	x	0	3	0
Melezitose	...	...	...	...	...	...							
Raffinose	...	...	...	x	4	3							
Dulcitol	0	...	...	...	...	...							
Glycerol	...	...	...	0	4	0							
Inositol	...	...	...	0	4	0							
Mannitol	x	...	...	x	4	4							
Salicin	x	...	...	x	3	1							

[a] 0 = negative; x = positive; 1, 2, 3, 4, = arbitrary designations of increasingly abundant acid, growth, and gas production.

rots, however, are described that affect cacti belonging to other genera. One rot with symptoms somewhat like those occurring in *Carnegiea* was studied by Pasinetti and Buzzati-Traverso (7) in *Cephalocereus senilis* in Italy. The rot was attributed to a bacterium isolated from the decaying tissues and proved pathogenic by inoculation; it was named *Bacterium cactivorum* Pasin. et Buzz.-Tr. sp. nov. The second rot, investigated by Johnston and Hitchcock (4), was found in flat opuntias growing in Florida. From the sick plants was obtained a bacterium pathogenic by inoculation in 7 species of prickly pear. This bacterium was designated *Bacillus cacticidus* Johnston and Hitchcock n. sp. A summarized comparison of the two bacteria with the bacterium from the giant cactus is given in tables 1 and 2.

DISCUSSION

The rot of *Cephalocereus senilis* described (7) by Pasinetti and Buzzati-Traverso is characterized by symptoms not unlike those of the necrosis of the giant cactus. In both diseases a dark discoloration accompanies the softening of the infected tissues. The decay in *Cephalocereus,* however, spreads so constantly from the base of the plant toward the apex that it has been termed ''humid basal rot''; in *Carnegiea* the rot proceeds from the infected spot in any direction that the parenchyma makes possible. Nor does the description of the pathogen, *Bacterium cactivorum*, agree with that of the pathogen, *Erwinia carnegieana.* The former, much smaller organism grows neither in peptone-dextrose nor in peptone-levulose and forms gas in malt broth; the reactions of the giant-cactus bacterium in those media are just the opposite. The limited extent of the reactions reported for *Bacterium cactivorum* make further comparison impossible; the data given for the pathogen are sufficient, we believe, to separate the two organisms.

The rot of the genus *Opuntia* attributed to the activities of *Bacillus cacticidus* is still more unlike the necrosis of the giant cactus than the rot of *Cephalocereus*, although, so far as symptoms are concerned, they are both ''wet'' rots. The pathogen isolated from the infected opuntias is smaller than *Erwinia carnegieana,* coccoid on solid media, gram negative, and may become yellow. More than 30 inoculations of *E. carnegieana* into *Opuntia* spp., hypodermically made at different times, failed. Moreover, opuntias abound in close proximity to necrotic giant cacti, yet none affected by a similar disease has been observed by us in the groves of southern Arizona. These conflicting data appear to be sufficient to remove the pathogen from our consideration as the cause of necrosis of the giant cactus.

Finally, the necrosis of *Carnegiea* and its pathogen, as well as the succulent nature of the host, remind the pathologist of vegetable soft rot and its cause, *Erwinia carotovora.* Both diseases affect parenchyma, both involve the dissolution of the middle lamella of the cell wall, both progress rapidly at high temperatures, and there are other similarities. However, the two pathogens differ in several respects,—size, grouping in cultures, usual reaction to Gram's stain, in milk, and in some carbon-source media. Cross-inoculation of the giant cactus with *Erwinia carotovora* gave negative results and numerous repeated inoculations of carrots and other hosts of the soft-rot organism with *Erwinia carnegieana* were likewise negative.

SUMMARY

A bacterial necrosis of the giant cactus (*Carnegiea gigantea* [*Cereus giganteus*]) is killing varying numbers of that plant over an extensive area in southern Arizona.

That the thinning of cactus groves now in progress may have been repeated in the past and may have continued until the affected groves became sparse or actually disappeared is suggested.

Macroscopic and microscopic features of the necrosis are described, also the cause, and a name is proposed for the pathogen.

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PHYTOPHTHORA PARASITICA ON PAPAYA (CARICA PAPAYA) IN HAWAII[1]

G. K. PARRIS

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INTRODUCTION

In March, 1940, a fruit- and stem-rotting disease of papaya was observed at Kahaluu and Kailua on the island of Oahu. At the former locality the disease was attacking approximately 40 per cent of the trees in a 4-acre planting and causing the death of the bearing portions of some 25 per cent. At Kailua, less damage occurred.

The cause of the disease was identified as *Phytophthora parasitica* Dastur, a diagnosis confirmed by C. M. Tucker.[2] A survey of the literature revealed that no investigator has presented a complete picture of Phytophthora rot of the papaya plant, and in only a few cases have Koch's rules of proof been applied to the causal agent.

LITERATURE REVIEW

Two species of *Phytophthora* have been reported attacking papaya in nature, *P. parasitica* (1, 11) and *P. palmivora* Butler (2, 3, 5, 6, 7, 8), and several unidentified species also have been recorded for this host. Tucker (10) determined the pathogenicity to papaya of a number of species of *Phytophthora,* including the two mentioned above, and found that not all strains of the same species were pathogenic and that a difference in pathogenic virulence existed between different strains. Mehrlich (4), Tucker (10), and Thung (11) showed that strains of *parasitica* would infect papaya only through wounds, and most investigators agree that infection by *palmivora* also takes place through wounds. Gadd (2), however, found that wound-free papaya fruits were infected by strains of the fungus from papaya and from rubber, while strains from other plants produced infection only after wounding.

SYMPTOMATOLOGY

Morphologic Symptoms. The terminal leaves on a diseased plant droop, wilt, dry out, or become yellowed, and fall prematurely. Eventually, only a few under-developed leaves persist at the apex of the plant. Fruits on a diseased plant may, also, fall prematurely. These symptoms (Fig. 1, A) are not unlike those exhibited by papayas suffering from foot (collar) rot or root rot, caused by various species of *Pythium,* particularly *P. ultimum* Trow (9, 12). In Hawaii root rot is caused by *P. aphanidermatum* (Eds.) Fitzpatrick.[3] Collar rot is infrequently caused by *Phytophthora parasitica,* but

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[1] Published with the approval of the director as Technical Paper No. 86 of the Hawaii Agricultural Experiment Station.

[2] Personal correspondence of December 19, 1940.

[3] Identified by J. T. Middleton; personal communication.

the roots of the papaya have not been observed to be attacked and diseased plants are firmly anchored in the soil. However, it should be noted (Table 1) that papaya roots are susceptible to the latter fungus, particularly when wounded.

With the disease under discussion, the symptom complex on the aerial parts of the plant results from one or more stem cankers, usually located in the upper third of the length of the stem. In their initial stages the cankers are small, $\frac{1}{2}$ to 1 in. in diameter, water-soaked, darker than normal, ovate to circular, and usually partly or completely covered with a whitish crust or mildew. The closely packed fruits on the stem of a bearing papaya tree may hide the cankers for a while, but as the cankers enlarge, fruits in their vicinity are attacked and fall, leaving one or more portions of the stem conspicuously bare (Fig. 1, B). The texture of a canker is rubbery, seldom soft; latex may or may not ooze from around the edges. With aging, the canker enlarges in both directions, but more rapidly along the length of the stem, until it may occupy an area of approximately 6″–8″ × 2″–4″. On a small plant, such a canker completely girdles the stem; on an older plant the stem above the lesion is seldom rotted or killed by the pathogen, but it is so structurally weakened that, in wind, it usually snaps off at the cankered zone.

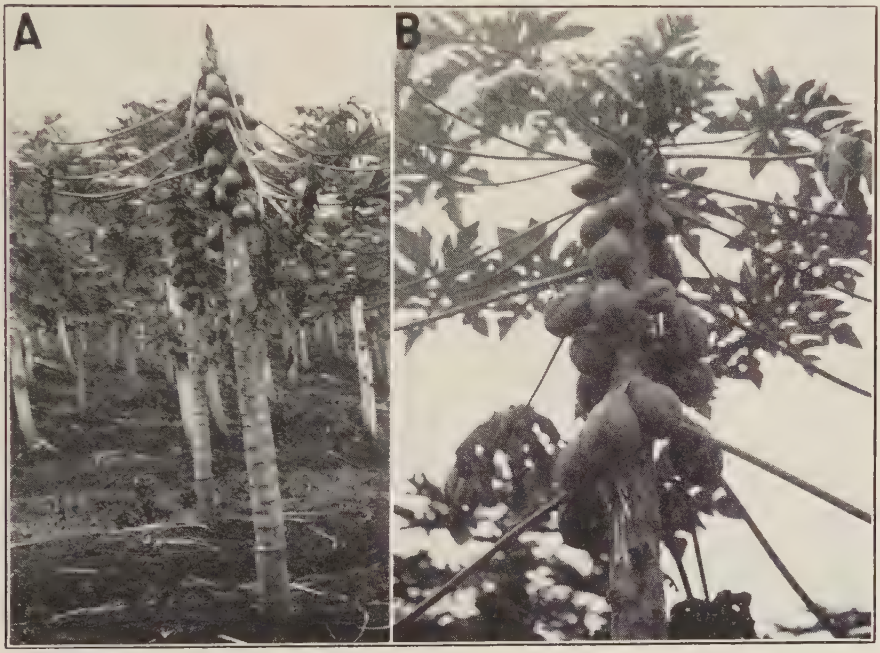

FIG. 1. Papaya trees diseased with *Phytophthora parasitica*. A. Top of plant killed following severe infection of stem in fruit-bearing region. Ground beneath tree shows numerous mummified fruits. B. Close-up of cankers on stem of less-severely affected tree than shown in A; note that many fruits and leaves have fallen prematurely and that the remaining foliage is wilting. Several fruits show white patches of mildew.

TABLE 1.—*Results obtained following inoculation of wounded and unwounded papaya leaves, stems, fruits, and roots with Phytophthora parasitica*

Point of inoculation		With or without wounding	With or without benefit of a moist chamber for 12 hours	Number of plants, or plant parts, inoculated	Number of plants, or plant parts, diseased	Type of infection		Number of infections from which fungus recovered
						Severe	Mild	
Stem	Upper third of length	Without	Without	4	0	......	......	......
			With	5	3	......	3	1
		With	Without	5	5	5	......	4
			With	1	1	1	......	1
	Middle of length	Without	Without	7	0	......	......	......
			With	5	5	......	5	2
		With	Without	6[a]	3	1	2	2
			With	1	1	1	......	1
	Lower third of length	Without	Without	5	0	......	......	......
			With	5	1	......	1	0
		With	Without	5	4	4	......	3
			With	1	1	1	......	0
Leaf	Upper surface	Without	Without	5	0	......	......	......
			With	5	5	5	......	1
		With	Without	5	5	......	......	......
			With	5	5	1	......	1
	Lower surface	Without	Without	5	0	......	......	......
			With	5	0	......	......	......
		With	Without	5	0	......	......	......
			With	5	1	......	1	0
Root		Without	Without	7	0	......	......	......
			With	4	1	......	1	1
		With	Without	6	1	......	1	0
			With	5	5	4	1	3
Fruit		Without	Without	10	0	......	......	......
			With	10	7	7	......	5
		With	Without	10	10	10	......	7
			With	10	10	10	......	8

[a] Five of these six plants were inoculated on wounds created by purposely breaking away a petiole of an old leaf.

As mentioned above, this disease infrequently causes cankers at the collar of the plant, that is immediately above the soil line. Here the organism meets with more resistance than when the stem proper is attacked. Even though an appreciable area may be killed, the plant usually survives but shows a semi-open cavity filled with dry, coarse fibres—the shredded and digested tissues of the stem. Immature plants may succumb to the collar-rot phase of this disease.

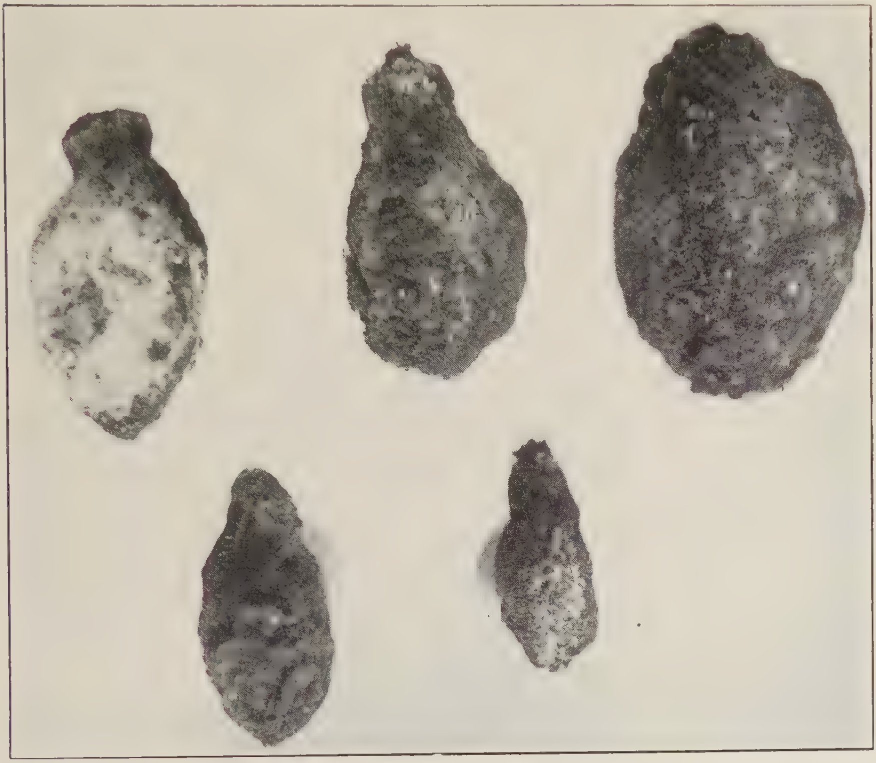

FIG. 2. Papaya fruits diseased and mummified following field infection by *Phytophthora parasitica.* Note the mycelium of the fungus fruiting on the surface of the mummies. Mummies are one-third to one-fifth size of originally healthy fruits.

Unlike the more common anthracnose rot of papaya fruits, caused by *Colletotrichum* sp. (probably *C. gloeosporioides*), Phytophthora rot causes the fruit to slowly shrivel, harden, and turn grayish-green. The surface may show one or more patches of a whitish incrustation, reminding one of powdery mildew (*Oidium* sp.). The mummified fruits ultimately become brownish-black (Fig. 2), light in weight, and of a stone-like texture. Fruits of all ages may be attacked.

Leaves were not observed to be diseased in the field, but were susceptible to artificial inoculation (Table 1).

Histologic Symptoms. Mycelium in diseased fruits is both inter- and intracellular. In newly diseased fruits circular, thin-walled, densely protoplasmic structures are found, sometimes attached to strands of mycelium (Fig. 3, A); in mummified fruits, oval or irregularly rounded chlamydospores with thicker walls are present (Fig. 3, B, C).

ETIOLOGY

The cause of this disease is *Phytophthora parasitica* Dastur, which produces its ovate, colorless, papillate, sporangia, attached to the host by short sporangiophores, on the surface of diseased parts. Placed in tap water at room temperature (27–28° C.), the sporangia germinate after the manner of a *Phytophthora*. One hundred sporangia from diseased fruits measured 32.0–38.4 μ by 22.4–25.6 μ, closely approximating dimensions given by Tucker (10) for *P. parasitica*. This worker has reported that *P. parasitica*, unlike *P. palmivora*, will grow at 35° C.; though it is significant to note that

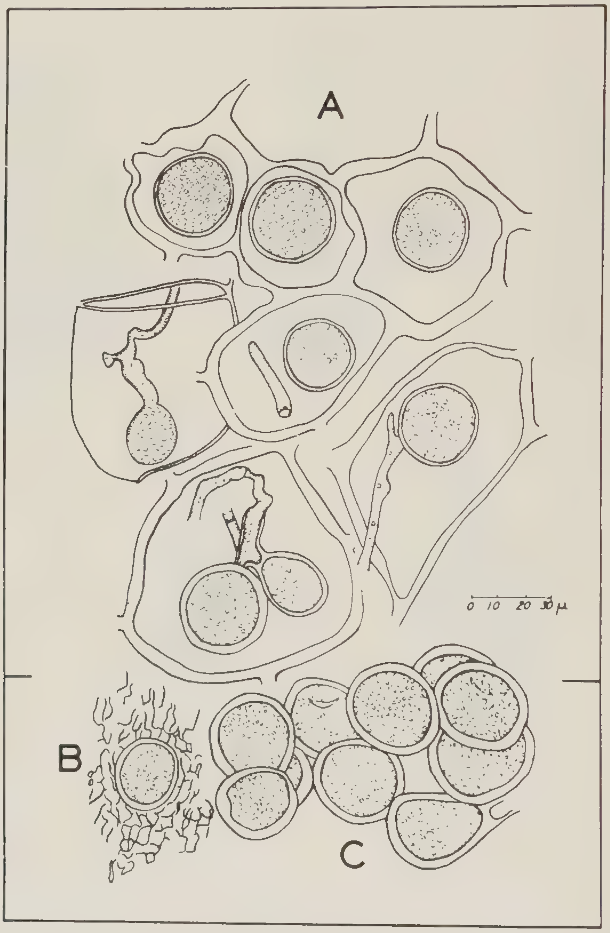

FIG. 3. Chlamydospores of *Phytophthora parasitica* in tissues of diseased papaya fruits. A. In freshly diseased fruits. B and C. In mummified fruits. In B the disorganized host tissue is pictured.

Mehrlich (4) found that certain strains of these species, isolated from pineapple in Hawaii, could not be separated from each other by growth or lack of growth at this temperature. Held at 35.5 ± 0.5° C. for 96 hours, the papaya isolate made no growth but the fungus was not killed; replaced at room temperature, all cultures soon developed abundant mycelium. Tucker[4] found that the Hawaiian isolate grew at 35° C.

Pathogenicity. The fungus was isolated in pure culture from diseased fruits and stems, and papaya leaves, stems, fruits, and roots were inoculated, with and without wounding, and with and without benefit of a moist chamber for 12 hours following inoculation. Wounds on stems, fruits, and roots were produced by a sharp scalpel; a small portion of plant tissue was removed, mycelium from an agar culture inserted, and the tissue replaced. Leaves were wounded by scarification with a needle. Following inoculation, plants were placed in a greenhouse where the temperature ranged from 75° F. (night) to 90° F. (day). In no case did any check plant, wounded or not, become diseased. Inoculations performed and the results obtained are presented in table 1. When infection occurred, attempts were made to reisolate and identify the pathogen; in many instances this was done, thus completing Koch's rules of proof.

No infection of uninjured parts occurred unless the plant was kept in a moist chamber following inoculation. In the absence of a moist atmosphere for a prolonged period, infection occurred readily when the fungus was introduced *via* a wound, except in the case of the lower surface of the papaya leaf where the concurrence of wounding and a high humidity was necessary for infection.

Young stems were girdled in 7 to 9 days, and fruits were covered with sporangia in 10 and mummified in 30 days, after inoculation. The appearance of leaf lesions was typical of Phytophthora blights; in 4 days the inoculated areas were necrosed, but there was no subsequent enlargement. The lower surface was more resistant than the upper surface of the leaf. Inoculated leaves abscissed prematurely. Root infection was relatively slow to affect the plant as a whole. After 2 to 2½ weeks, the leaves of inoculated plants fell prematurely and the stem was anchored less securely in the soil than is normally the case. Subsequently, plants inoculated on the roots toppled over or could be easily uprooted. The tap root (inoculated) was softened and somewhat decomposed and few laterals were alive. These plants often died in another week to 10 days, but sometimes the plant slowly recovered and continued to grow with the stem in a semi-recumbent position. Inoculations at the collar produced a similar response.

CONTROL

Growers decapitate trees as soon as cankers appear on the stems, and collect and destroy diseased material. Decapitation saves time; such trees produce fruits sooner than do replants. Sooner or later, a certain percentage of the new shoots that develop are diseased, and the decapitation procedure is repeated.

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[4] Personal correspondence.

Spraying the papaya plant with a copper fungicide undoubtedly would control Phytophthora rot, but, so far, no attempt has been made to protect the plants. Local data have shown that Bordeaux mixture causes russetting of fruits, burning of the young foliage and a stunting of the papaya plant. Cuprocide 54–Y causes no comparable damage.

SUMMARY

A disease of papaya (*Carica papaya*) in Hawaii, which causes hard rotting of fruits and cankers on the stem, is described. Diseased fruits fall prematurely and mummify, while cankered stems may be girdled and distal parts die from lack of water and nutrients; or the stem may snap off in the wind at the cankered zone. Symptoms on the plant as a whole resemble those of root rot of papaya (*Pythium* spp.); but, in the field, the roots are not thought to be attacked by the cause of the disease, which is *Phytophthora parasitica* Dastur.

The fungus is essentially a wound parasite, though it will infect wound-free stems, fruits, roots, and leaves if the plant be kept in a moist chamber following inoculation. In a large number of instances the fungus was recovered and identified from artificially induced lesions.

Growers decapitate diseased trees, since beheaded plants produce fruits sooner than do replants. A copper fungicide probably will control the disease, but has not been tried. Bordeaux mixture russets papaya fruits, burns young foliage, and stunts the plant; Cuprocide 54–Y does not produce these harmful effects.

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THE EFFECT OF TEMPERATURE ON SYMPTOM EXPRESSION OF A ROSE MOSAIC[1]

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(Accepted for publication July 7, 1941)

The expression of symptoms of rose mosaic is recognized as being erratic under even favorable environmental conditions (2, 3, 9). Symptoms may be exhibited in some leaves or leaflets, while adjacent ones frequently are normal in appearance. It is practically impossible accurately to diagnose the disease in named varieties, growing outdoors, because of the mildness or lack of leaf symptoms and the presence of injuries from insects, fungi, chloroses, and genetical leaf markings (2, 3, 6). In rootstock varieties the situation in the field is even worse. There is no convincing evidence that field diagnosis of this mosaic in rootstock or bud varieties is possible on a commercial scale.

Plants, apparently mosaic-free when growing outdoors, may develop striking symptoms when moved into the greenhouse; but leaves formed after being returned outdoors will be normal. On April 4, 1940, 12 plants of each of 2 varieties, Peerless and Rome Glory, all of which showed typical symptoms in the glasshouse, were pruned and defoliated before being moved outdoors. Positive symptoms were not shown by any of the abundant new leaves produced during the next 5 months. Two plants of each variety, handled similarly but kept in the glasshouse, continued to show typical symptoms. White (8) reported similar observations.

Temperature is one of the environmental factors that may be involved in this difference in symptom expression outdoors and under glass. This relationship was investigated in studies reported in the present paper.

MATERIALS AND METHODS

Infected plants of the Peerless and Rome Glory varieties were selected in commercial greenhouses where they had been growing 2½ to 3 months. These plants were placed in 10-inch pots and transferred to a greenhouse in Berkeley for 37 days to recover from transplanting shock. Each pot was mulched with well-rotted manure and, during the experiments, occasionally watered with a dilute solution of potassium sulphate and ammonium sulphate.

The 12 plants of each variety that showed most conspicuous symptoms were transferred to 2 controlled-environment chambers[2] for the experiment. In the 3 series of tests the atmosphere was held at a saturation deficit approximately equal to a relative humidity of 70 per cent at 17° C. (*i.e.*, 4.3

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[1] This study is limited to the disease which produces symptoms of the type commonly seen in greenhouses (2, Figs. 1 and 2; 3, Figs. 1, A, and 1, C; 6, Fig. 1; 9, Figs. 1, B, and 3, A).

[2] Equipment was elaborated and improved by Davis and Hoagland over that described in an earlier paper (4), and was kindly made available for these studies by the Division of Plant Nutrition, University of California.

g. water per cu. m. of air would produce saturation at 17° C.). The plants received no sunlight, but were illuminated for 10 hours each day with 20 24-inch fluorescent tubes inside each chamber, 13 being pink and 7 blue. Because growth was somewhat spindling under these conditions in the first series, supplemental illumination of 4 incandescent 300-watt bulbs with reflectors was placed outside each glass-walled chamber during series 2 and 3. Duration of the daily light period also was 10 hours in these series.

In series 1 the 2 chambers were held continuously for 37 days at 17° and 25° C., respectively. The latter temperature under these constant conditions was too high for good growth of these varieties and caused partial defoliation. However, plants from these 2 temperature tests showed approximately equal growth in the next series. In series 2 one chamber was operated at 25° C. for the 10-hour light period and at 19° C. during the dark period. The other chamber was operated similarly at 18° day and 12° C. night temperatures. In series 3 one chamber was kept continuously at 21°, while the other was alternated from 21° day to 15° C. night temperature. The duration of the second series was 56 days and of the third, 62 days. The plants were redistributed in the chambers at the beginning of the second and third series to compensate for possible effects of previous environment. However, no such effects were noted on either symptoms or growth.

At the beginning and end of each series separate counts of mosaic and normal leaves, and measurements of growth were made on each shoot. These are presented in table 1 for each of the series.

TABLE 1.—*Effect of temperature on symptoms of rose mosaic in the Rome Glory and Peerless varieties*

Variety	Series	Temperature (°C.)		Shoot growth (cm.)	New leaves	
		Night	Day		Number	Mean per cent with mosaic[a]
Rome Glory	1 a	17	17	124.0	112	44.53 ± 5.24
	b	25	25	165.3	124	36.48 ± 4.33
	2 a	12	18	146.1	113	26.79 ± 4.58
	b	19	25	356.3	140	40.38 ± 3.57
	3 a	15	21	442.0	175	13.58 ± 2.98
	b	21	21	937.2	349	27.67 ± 2.76
Peerless	1 a	17	17	244.1	137	63.99 ± 4.07
	b	25	25	106.6	89	56.19 ± 6.37
	2 a	12	18	200.5	179	18.27 ± 2.77
	b	19	25	181.6	113	41.08 ± 5.23
	3 a	15	21	962.1	266	35.73 ± 3.81
	b	21	21	690.3	200	51.53 ± 3.69

[a] Estimated standard error of the mean was computed by the formula,

$$\sigma_x = \frac{1}{n}\sqrt{\frac{p_1q_1}{m_1}+\frac{p_2q_2}{m_2}+\ldots\frac{p_nq_n}{m_n}},$$

in which n is the number of plants (6 in each case), m the number of new leaves on a given plant, p the probability of a leaf showing mosaic symptoms, and $q = 1 - p$. Since these true probabilities, p and q, are unknown for a particular plant, the estimates based on observations are the best substitutes for them. In this analysis p and q were expressed as the percentage of mosaic and normal leaves, respectively.

RESULTS AND DISCUSSION

In these tests Rome Glory exhibited consistently better growth at the higher of the two temperatures in each series, and Peerless showed better growth at the lower range, although the varieties were intermingled and grew under very similar conditions. Despite this dissimilar growth trend, there was definite consistency in the relationships of symptom expression and temperature exhibited by the two varieties. This suggests that the response to temperature is a result of action on the virus rather than on the host. Beyond the minimum requirement of production of some vigorous new leaves, there was no observed relation between vigor of growth and severity of symptoms exhibited.

In series 1 the symptoms at 17° were strong and leaflets often were badly distorted, with conspicuous white markings; those at 25° C. were less severe. In series 2 the symptoms in both varieties were definitely suppressed in the low-temperature chamber of 12° and 18° C. In the third series symptoms were likewise suppressed in the chamber at 15° and 21° C. Because the severity of the symptoms was observed to increase with the percentage of leaves affected in any given series, the latter numerical index of disease severity was used.

Plants of the Peerless variety grown at 12° at night and 18° C. during the day, and those on a 15° and 21° C. schedule, had markedly lower percentages of mosaic leaves than the plants grown at the other temperatures (Table 1). Rome Glory grown at a night temperature of 15°, and 21° C. during the day, had significantly lower percentages of mosaic leaves than those held at other temperatures, and the trend was the same for those on the 12° and 18° C. schedule, although the differences were not so great.

There was considerable fluctuation in percentage of mosaic leaves in other series, but the levels were higher than for the two series mentioned above. It is quite possible that, with larger populations of plants, a more definite optimum for expression of mosaic symptoms would be found, but the important point here is that unmistakable symptoms are produced over a considerable range of temperatures. The severity and incidence are reduced conspicuously only by temperatures of 15° C. or less; but these temperatures apparently need not be continuous to be effective.

Even under the carefully controlled environmental conditions of the series held continuously at a favorable temperature for symptom expression, there was no consistency in the distribution of leaves showing the disease. A given leaf adjacent to another that showed mosaic might or might not exhibit symptoms, and even companion leaflets were frequently dissimilar in this respect. The virus is either extremely sensitive to the micro-environment of the leaf, an unlikely condition in view of the range of favorable temperatures, or some unknown factors in the physiology of the leaf or the movement of the virus are involved.

The inhibiting effect of low temperatures on the mosaic may account in part for the usual absence of symptoms outdoors, but probably is not the sole factor involved. Under coastal California conditions the temperature during the active growing season sometimes stays within the limits for expression of symptoms for periods of 2 or 3 weeks, but the disease is almost never seen in the field.

Roses usually are grown commercially in glasshouses at temperatures of 14.5°–17° C. at night and, when possible, 20°–25° C. in the daytime. These ranges are such that temperatures under glass would be almost continuously conducive to expression of symptoms. For that reason it is possible to identify vigorously growing infected plants with certainty in the glasshouse and, if desired, rogue them out.

RELATION OF SYMPTOM EXPRESSION TO CONTROL OF THE DISEASE

Roguing in a glasshouse is justifiable if the flowers of the variety are sufficiently injured or reduced in number (3, 10) that the infected plants do not return a profit. The plants adjacent to those removed will benefit from the increased space, light and lessened soil competition, or if roguing is done early the beds can be replanted.

There apparently is little reason to fear that the disease will spread under glasshouse conditions, and infected plants need not be removed because of that consideration alone. As early as 1930 White (8) stated that "Due to its apparent failure to spread in the Eastern greenhouses, the commercial grower has rogued out diseased individuals very successfully." Beds having up to 50 per cent of infected plants in 1928–29 were rogued in the summer of 1929, and in 1930 diseased plants were either absent or present only in very small numbers. He later (10) found that 8 out of 36 seemingly healthy plants in a greenhouse test developed mosaic in the first 3 months, but no more appeared in the following 21 months. Presumably growth was insufficiently vigorous in the first 3 months for reliable diagnosis. Berkeley (1) found that after removing the infected 50 per cent of Premier rose plants in a Toronto greenhouse, "very few diseased plants were observed after 3 months' time."

TABLE 2.—*Incidence of mosaic in several rose varieties in glasshouses at two dates, as a measure of spread of the disease*

Variety	Number of plants	Initial age of planting	Interval between observations	Per cent plants with mosaic	
				Initial	Final
		Months	*Months*		
Better Times[a]	84	22	9	22.6	22.6
Peerless[b]	204	11	9	13.2	13.7
Hollywood	119	24	25	16.0	16.8
Rome Glory	70	2½	8	42.9	47.1

[a] Examination made 4 months after planting showed 11.9 per cent mosaic; this reading was later found to be inaccurate, as some of the plants had showed insufficient new growth for accurate diagnosis.

[b] Three months after planting had only 7.4 per cent mosaic detectable for the same reason indicated for Better Times.

General observations and successive counts in California commercial glasshouses at intervals up to 2 years have given no clear evidence of the spread of rose mosaic. These data are summarized in table 2. The slight increases noted are explainable by the fact that the growth status of some of the plants at the time of early observations made accurate diagnosis difficult; this is shown in the readings taken 2½–4 months after planting. The disease certainly cannot be regarded as dangerously infectious in greenhouses and this interpretation is reflected in the present attitude of commercial growers toward it.

It is interesting that the disease, practically from the time of its first clear recognition, has been regarded as not spreading in the glasshouses. However, until comparatively recently (2, 3, 6), the prevalent view has been that natural spread did occur in the field (5, 7, 8). Because of the admitted difficulties of field, as compared with glasshouse, diagnosis this situation is not hard to understand when the relative dependability and severity of symptoms in the two locations are considered. However, as pointed out by Brierley and Smith (2, 3), the nursery practices of propagating understocks from the tops of previously budded plants, and of using budwood from top varieties growing outdoors, probably account for known field "spread" of the disease. When buds are taken in glasshouses, there is the hazard that they will be taken from the larger, nonproductive, mosaic-infected plants rather than from the much-pruned healthy ones, particularly if the nature and appearance of the disease are not understood (6).

For varieties that suffer flower deformation or yield reduction from the disease, it would be well to select budwood from greenhouse plants that have been examined at intervals and found mosaic-free. Before starting large-scale propagation of a new greenhouse variety, nurserymen could well grow the plants for budwood under glass at temperature favorable for the detection of the disease. This selected material could then be increased in mother blocks in the field.

Whenever necessary to increase the supply of budwood in the field it would be a reasonable precaution to plant at some distance from any infected nursery stock.

SUMMARY

Symptoms of mosaic were found in tests with two rose varieties growing under conditions of controlled environment to be favored by temperatures of 15–25° C. and to be greatly reduced in severity only below 15° C.

Under carefully controlled environmental conditions, as under fluctuating glasshouse situations, there was an erratic appearance of symptoms in comparisons between adjacent leaves or companion leaflets.

The response of the varieties to temperature was consistently distinct in the matter of growth, but in mosaic symptom-expression it was the same.

Because of the wide limits of the favorable temperature range for mosaic symptoms, it should be possible for certain purposes to grow satisfactory

budwood under glasshouse conditions, where diseased plants can be recognized and eliminated, and where the danger of new infections is slight.

There was no evidence of spread of the mosaic in commercial greenhouses.

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PHYTOPATHOLOGICAL NOTES

Pythium arrhenomanes on Cereals and Grasses in the Northern Great Plains.[1]—On the basis of pure-culture isolations made from field-grown cereals and grasses in North Dakota in 1940 and 1941, the common root-browning organism, *Pythium arrhenomanes* Drechsl., appears to be prevalent. While it seems to be particularly important in the lighter soils of central North Dakota, it is also abundant in the Red River Valley and has been isolated from the northern, extreme western, and northeastern parts of the State with frequent regularity. Isolations have been made also from wheat, corn, and certain grasses in adjacent Minnesota and South Dakota, and from wheat in eastern Montana. Strains of *P. aristosporum* Vanterpool, another species strongly pathogenic to cereals and grasses, which is occasionally isolated from wheat affected with browning root rot in Saskatchewan,[2] were obtained from barley roots from Dickinson, North Dakota, and from wheat roots from Bozeman, Montana.

During 1941, the disease first appeared about May 10, on various hosts, and continued to develop until about July 10. *Pythium arrhenomanes,* however, was isolated from such warm temperature grasses as *Setaria italica* (L.) Beauv. until early August, and in the Red River Valley was isolated from wheat as late as July 29. Other species of *Pythium,* including *P. debaryanum* and several congeneric forms, were isolated also from damping-off grasses early in the season and, to some extent, later, following the rainy periods.

Pure cultures of *Pythium arrhenomanes* isolated from a number of hosts proved parasitic on Thatcher wheat seedlings grown under aseptic conditions in small Erlenmeyer flasks held at room temperatures at Saskatoon, in August, 1941, and at Mandan, N. Dak., in September, 1941. Cultures from the following field-grown, naturally infected hosts were moderately to severely parasitic on the wheat seedlings: *Aegilops triuncialis* L., *Agropyron amurense* Drob., *A. caninum* (L.) Beauv. (the seed of this lot came originally from Russia and appears to be true *A. caninum* and not *A. caninum* Amer. Auth., which latter, according to Hitchcock, is *A. subsecundum*), *A. ciliare* (Trin.) Franch., *A. cristatum* (L.) Gaertn. (standard), *A. cristatum* (rhizomatous form), *A. dasystachyum* (Hook.) Scribn., *A. intermedium* (Host) Beauv., *A. pungens* (Pers.) Roem. and Schult., *A. repens* (L.) Beauv., *A. trachycaulum* (Link) Malte (*A. pauciflorum*), *Ammophila arenaria* (L.) Link, *Bouteloua curtipendula* (Michx.) Torr., *B. gracilis* (H.B.K.) Lag., *Brachypodium sylvaticum* (Huds.) Beauv., *Bromus carinatus* Hook. and Arn., *B. erectus* Huds., *B. inermis* Leyss., *Echinochloa crus-*

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[1] Cooperative investigations between the Divisions of Cereal Crops and Diseases, Forage Crops and Diseases, and Dry Land Agriculture, Bureau of Plant Industry; and the Division of Nurseries, Soil Conservation Service, U. S. Department of Agriculture; the North Dakota Agricultural Experiment Station; and the Plant Pathology Laboratory of the University of Saskatchewan, Saskatoon, Canada.

[2] Vanterpool, T. C. Present knowledge of browning root rot of wheat with special reference to its control. Sci. Agr. 20: 735–749. 1940.

galli (L.) Beauv., *Elymus glaucus* Buckl., *E. interruptus* Buckl., *E. junceus* Fisch., *Festuca rubra* var. *commutata* Gaud., *Hordeum vulgare* L., *Panicum miliaceum* L., *Secale cereale* L., *Setaria italica* (L.) Beauv., *S. viridis* (L.) Beauv., *Stipa comata* Trin. and Rupr., *Triticum aestivum* L., *T. dicoccum* Schrank, *T. durum* Desf. and *Zea mays* L. Under the same conditions of experimentation, many of the sphaerosporangial forms obtained from damping-off grasses proved to be slightly, and less often moderately, parasitic on wheat, while others showed no signs of parasitism.

In addition to the hosts listed above, the following species have yielded *Pythium arrhenomanes* from diseased field-grown, naturally infected plants, but the parasitic nature of these isolates remains to be proved: *Agropyron desertorum* (Link) Richt., *A. riparium* Scribn. and Sm., *A. spicatum* (Pursh) Scribn. and Sm., *A. trichophorum* (Link) Richt., *Arrhenatherum elatius* (L.) Mert. and Koch, *Avena fatua* L., *A. sativa* L., *Sorghastrum nutans* (L). Nash, *Sorghum vulgare* Pers., and *S. vulgare* var. *sudanense* (Piper) Hitch. Since all of the isolates of *P. arrhenomanes* tested on wheat were definitely pathogenic, it is believed that most or all of the untested cultures of this fungus will prove similarly so.

It would appear from the foregoing results that damage to cereals and grasses from *Pythium* is just as common and serious in the Northern Great Plains as in Saskatchewan and that, as has been previously pointed out, the parasitic species concerned are indigenous on our native grasses.

A more detailed host-range and list of isolates, together with data on other species of *Pythium* besides *P. arrhenomanes,* will be assembled later.—T. C. VANTERPOOL, University of Saskatchewan, Saskatoon, Canada, and RODERICK SPRAGUE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Northern Great Plains Field Station, Mandan, N. Dak.

Savoy Disease of Sugar Beets in Southwestern Ontario.[1]—In the course of periodic surveys of sugar-beet fields of southwestern Ontario during the current season, some hundreds of plants exhibiting symptoms suggestive of disease of the virus type were marked for special observation. In the case of certain of the suspect plants the cause and nature of their apparently diseased condition remain obscure. In the case of others, however, the symptoms exhibited were identical in every respect with those described by Coons and co-workers[2] in 1937, for Savoy, a virus disease of beets transmitted by the pigweed bug, *Piesma cinerea.* The latter insect, which has a wide host range including the red root pigweed and other members of the pigweed family, has been reported by Stirrett[3] as occurring in the sugar-beet fields in Ontario. Figure 1 shows a severe case of Savoy, infection having reached the stage of total involvement of the leaves of a 5-month-old sugar beet.

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[1] Contribution No. 686 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Coons, G. H., J. E. Kotila and Dewey Stewart. Savoy, a virus disease of beet transmitted by *Piesma cinerea.* Phytopath. (Abstract) 27: 125. 1937.

[3] Stirrett, Geo. M. A contribution to the knowledge of sugar beet insects in Ontario. Sci. Agr. 16: 180–196. 1935.

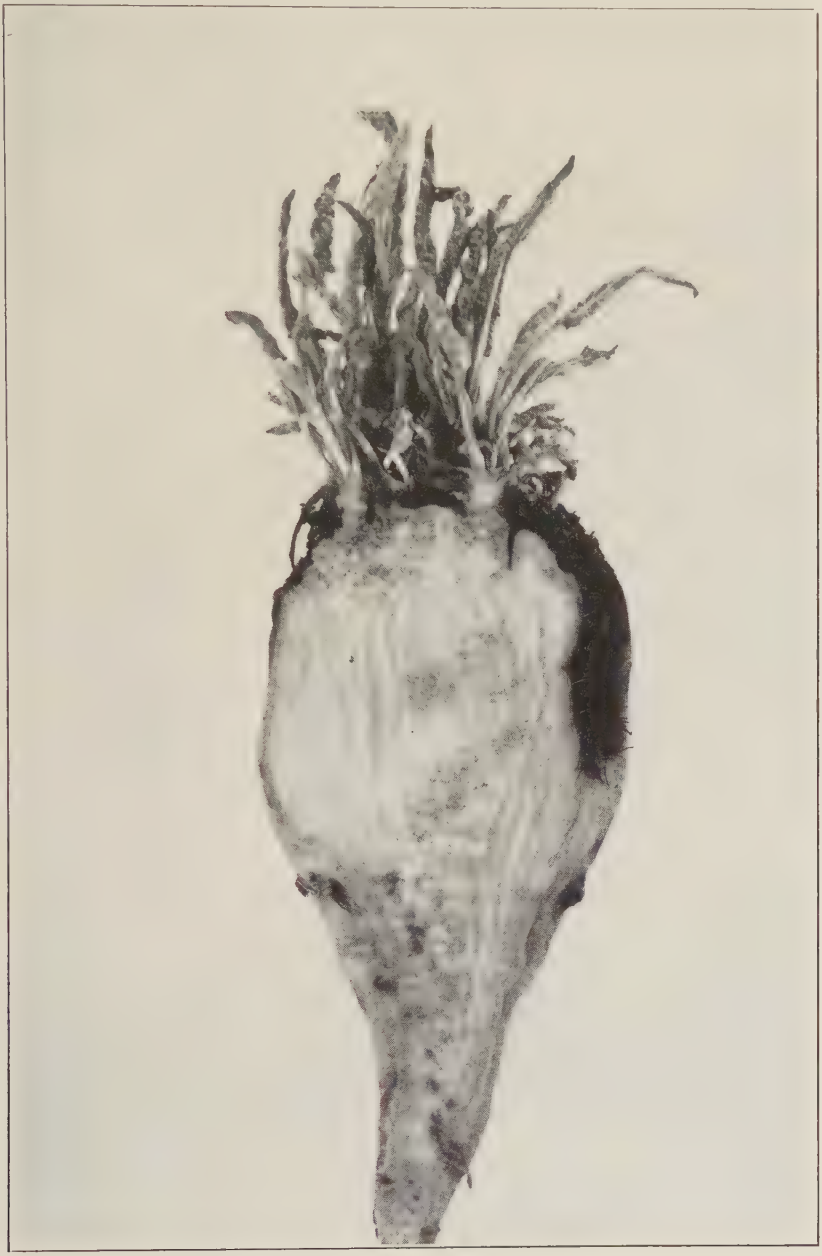

FIG. 1. Five-month-old sugar beet showing typical, late-stage symptoms of Savoy disease. × $\frac{2}{3}$.

As far as the writers are aware, this disease of beets has not been reported heretofore in Ontario, but it is known that in October, 1939, H. D.

Brown of the Agricultural Research Department of the Canada and Dominion Sugar Company, Chatham, Ontario, had "Savoy" plants under observation.

In most of the fields examined this year only a trace of the disease was found. However, in several fields in one particular district, the number of affected plants reached proportions that must have been of appreciable economic significance. The circumstances in connection with the incidence of affected plants in one of these fields seem of sufficient interest to warrant consideration in the following detail. Commencing with the outside row on one side of the field and proceeding row by row towards the center, 11,200 plants were counted in successive groups of 100 within each row (Fig. 2).

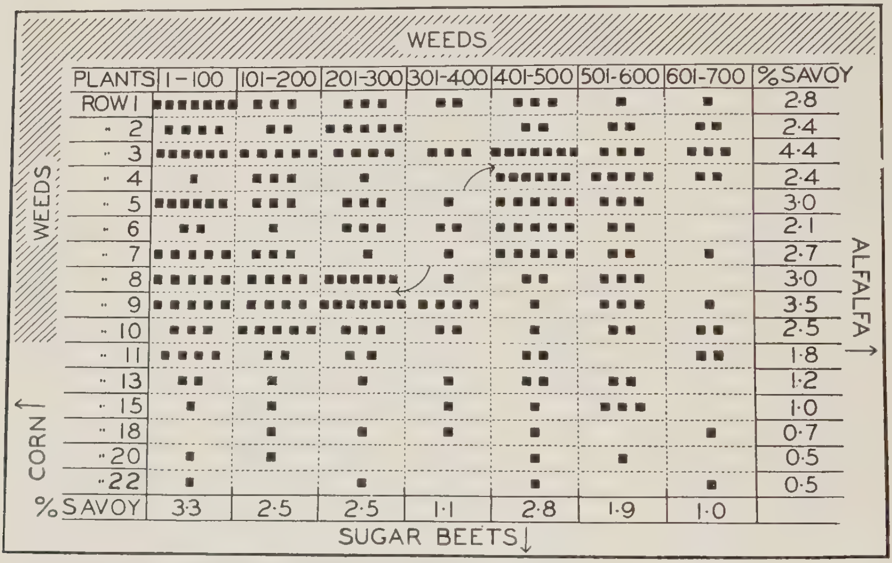

FIG. 2. Diagram showing correlation between occurrence of sugar beets infected with Savoy disease and presence of weeds in close proximity.

Altogether, 245 plants, that is, 2.1 per cent, showed definite symptoms of Savoy. Analysis of the data of the completed count indicated that infected plants were not distributed evenly throughout the field. Rather, it was noted that infection was greater in one corner and along one side of the field than towards the center. For example, the percentage occurrence of the disease in rows 3, 11, and 22, was 4.4, 1.8, and 0.5, respectively. In the first 10 rows the number of infected plants averaged 2.6 per cent, whereas in the next 6 rows counted (11, 13, 15, 18, 20, and 22), the average dropped to 0.9 per cent. In the areas indicated by the arrows in figure 2, infection reached a maximum of 6.5 per cent.

Along the one side and at the corner of the field towards which infection was highest, weeds were growing in profusion. Among the latter *Ambrosia artemisiifolia* greatly predominated, but there were also present *Daucus carota*, *Solidago canadensis*, *Chenopodium album*, *Trifolium repens*, *Amaranthus* spp. and others in lesser numbers. Circumstantial evidence would suggest that some of the weeds mentioned above were infected with the virus transmitted to the beets following invasion of the field by viruliferous insects.—A. A. HILDEBRAND and L. W. KOCH, Laboratory of Plant Pathology, Harrow, Ontario.

Mosaic of Bromus inermis.—During the spring of 1941, the writers collected a yellow mosaic on *Bromus inermis* Leyss. growing in the headland of the wheat rust nursery at the Kansas Agricultural Experiment Station, Manhattan, Kansas. In a limited survey in the vicinity of Manhattan, no other case of mosaic on *B. inermis* was found.

Infected plants from Manhattan were transported to Arlington Farm, Virginia, and inoculation studies were carried out. *Bromus inermis,* Harvest Queen wheat, and White Tartar oats were found susceptible, developing severe yellow-mosaic symptoms. Infected tissue was clipped, dried, and stored at summer room-temperatures and, at intervals, tested for potency in wheat. At the end of 51 days, when the last test was made, there was sufficient active virus to induce mosaic in all of the 12 wheat plants inoculated. Further tests may show the period of potency to be still longer.

Bromus inermis was inoculated with each of the 7 wheat viruses previously listed,[1] and the Bromus virus was used as a control on the technique. Each virus was inoculated into 20 healthy plants of *B. inermis.* The Bromus virus induced typical mosaic in 16 plants, but none of the wheat viruses induced symptoms. Inoculations from these symptomless plants have failed thus far to indicate any virus carriers. It is concluded that the Bromus virus is distinct from the other viruses tested.—H. H. MCKINNEY, H. FELLOWS, and C. O. JOHNSTON, Bureau of Plant Industry, U. S. Department of Agriculture.

A Convenient Scale for Use in the Rapid Determination of Comparative Degrees of Infection of Hops by the Downy Mildew Fungus, Pseudoperonospora humuli.[1]—Moist chambers are prepared, in the course of laboratory inoculation experiments, by placing moistened discs of filter paper in the bottoms of Petri dishes. Excised leaves of hop (*Humulus lupulus* L.) are placed on the filter paper, as shown in figure 1, and distilled water suspensions of the zoosporangia of the downy mildew fungus (*Pseudoperonospora humuli* (Miyabe and Tak.) Wilson) are atomized, with a DeVilbiss atomizer, onto the exposed lower leaf surfaces. The covers are placed on the dishes and the moist chambers then removed to an incubation cabinet maintaining a constant optimum germination temperature of approximately 65° F. After an incubation period of suitable length infection of the leaves

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[1] McKinney, H. H. Mosaic diseases of wheat and related cereals. U. S. Dept. of Agric. Cir. No. 442, 22 p. 1937.

[1] Published as Technical Paper No. 405 with the approval of the Director, Oregon Agricultural Experiment Station—Reporting a cooperative project between the U. S. Bureau of Plant Industry and the Oregon Agricultural Experiment Station.

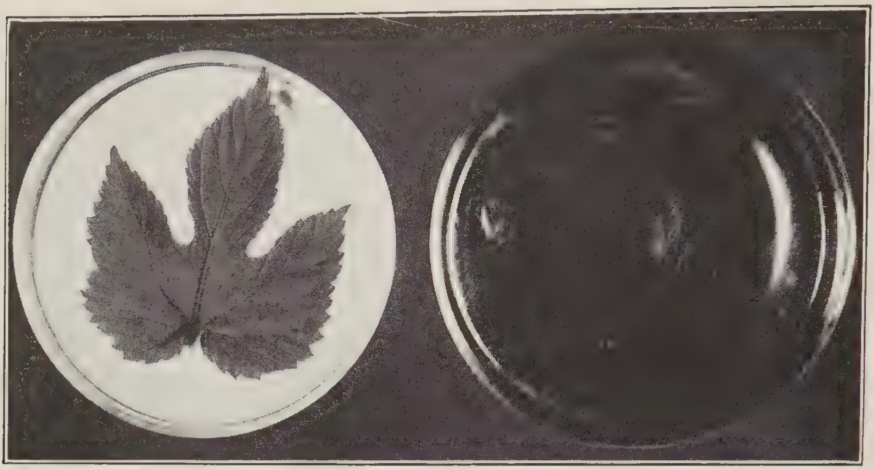

FIG. 1. A Petri-dish moist chamber with cover removed to show hop leaf in place.

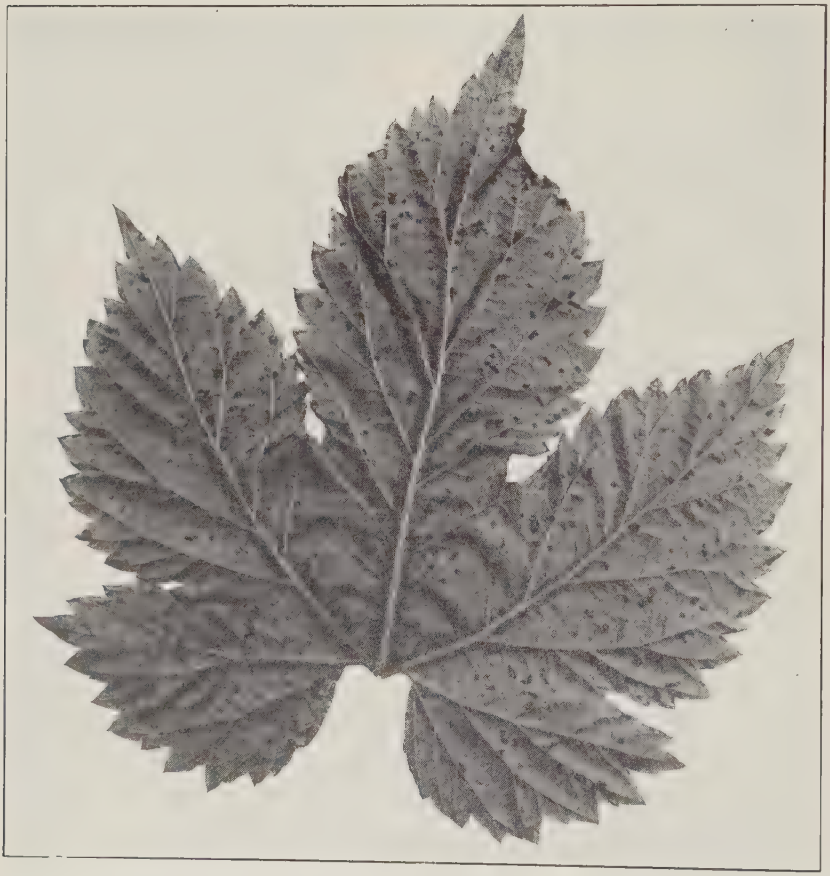

FIG. 2. Typical secondary leaf infection resulting from natural infection in the field, of Late Clusters, a susceptible variety of hops.

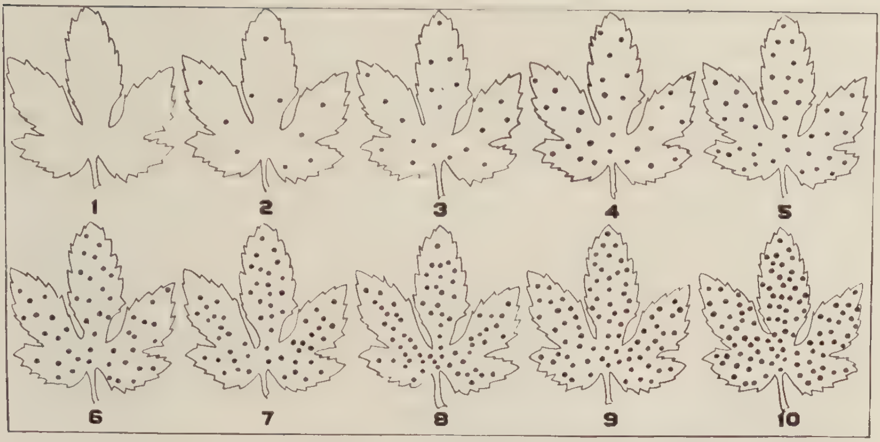

FIG. 3. Scale showing comparative degrees of leaf infection.

of susceptible hosts is evidenced by the appearance of clusters of sporangiophores bearing dark-colored sporangia as shown in figure 2.

The scale found to be convenient for use in the rapid determination of comparative degrees of infection is illustrated in figure 3. It was prepared by tracing the outlines of a hop leaf of convenient size to fit into the Petri dish moist chamber and by placing within each outline a definite number of dots, from 0 to 100, to represent points of infection. Numerals from 0 to 10 are used to represent the comparative degrees of infection. By reference to the scale numerical values can rapidly be assigned to the infection appearing on the leaves as they are removed from the moist chambers, particularly if the leaves are examined with the aid of a watchmaker's loupe.—G. R. HOERNER, Division of Drug and Related Plants, Bureau of Plant Industry, United States Department of Agriculture, Oregon State College, Corvallis, Oregon.

The Rasp Leaf of Cherry.[1]—A disease of cherry trees manifesting itself by abnormalities of the leaf growth was observed first in 1935 on the Royal Ann variety at Cedaredge in Delta County, Colorado. In 1938 cherry trees of the Bing, Lambert, and Royal Ann varieties near Paonia, Delta County, were found affected with the malady. Observations in 1940 and 1941 indicated a quite rapid spread of the disease in this district. The Royal Ann variety appeared to be most severely affected.

The enations, which develop on the underside of the leaves, constitute the most characteristic symptom of the disease and vary from elongated protuberances to raised, serrate, leaf-like growths (Fig. 1, A, D). Usually a gland is present at the apex of each leaf-like growth (Fig. 1, D). On a given leaf the growths usually are found spreading outwardly from the midrib between the veins toward the margin of the leaf blade. The upper surface of the leaf blade shows depressed rugosities, of lighter color than the normal green of the leaf (Fig. 1, C). In all cases such ventral depressed and discolored areas are directly opposite a dorsal outgrowth. Because the majority of the dorsal outgrowths resemble the teeth of a coarse rasp and because this is the major manifestation of the disease, the name rasp-leaf is suggested to designate the disease. Severely affected leaves are small, nar-

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[1] Published with the approval of the Director, Colorado Agricultural Experiment Station, as Scientific Series paper #131.

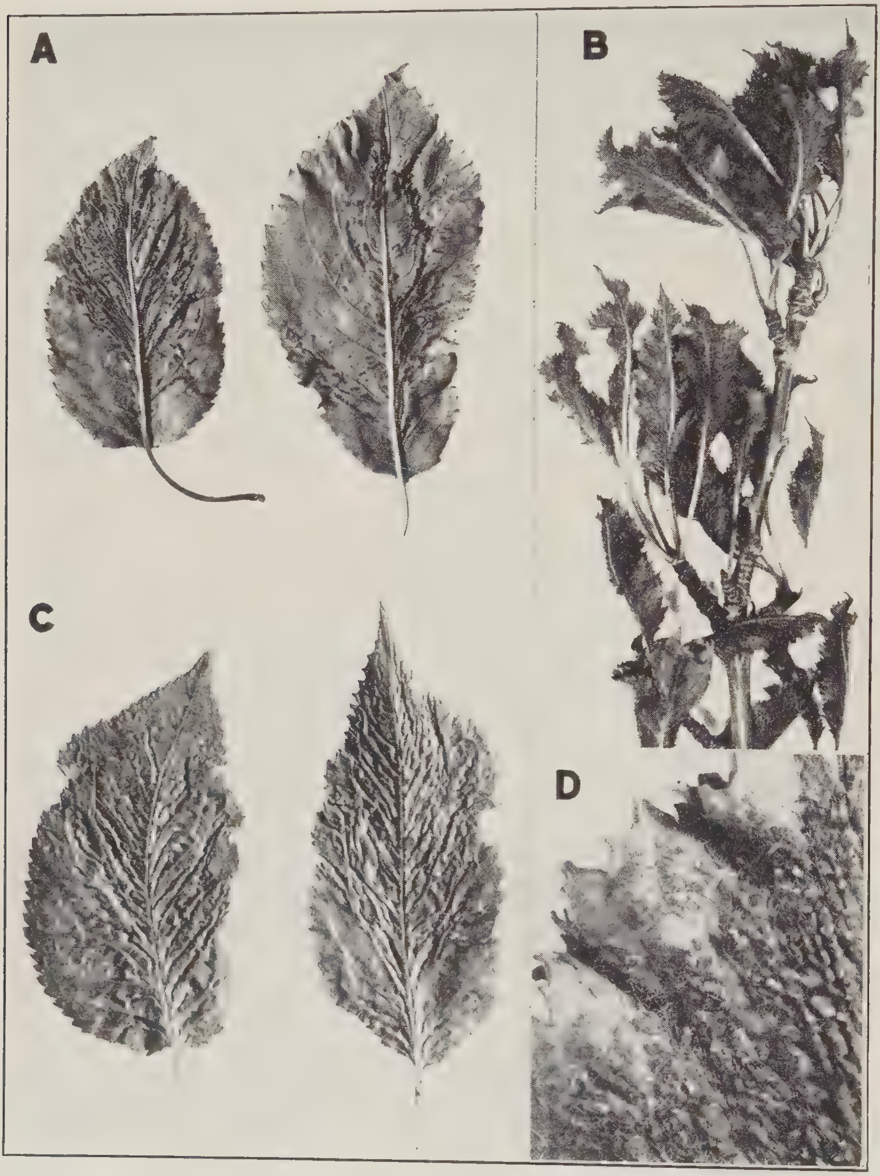

FIG. 1. Symptoms of rasp leaf of cherry on the Royal Ann variety. A. Enations on lower surface of affected leaves. B. Diseased twig showing distortion and ventral folding of the leaves. C. Ventral surface of affected leaves showing depressed rugose areas. D. Enlarged portion of dorsal surface of diseased leaf showing outgrowths. In many instances an apical gland may be noticed.

row, and markedly distorted. The leaf blade frequently shows a tendency to fold upon itself ventrally (Fig. 1, B). The damage to the tree apparently results from the retardation in growth.

Bud-inoculation tests to date indicate that the disease is of a virus nature, with a two-year incubation period.—E. W. BODINE, Section of Botany and Plant Pathology, Colorado Experiment Station, and J. H. NEWTON, Bureau of Plant and Insect Control, Colorado State Division of Agriculture.

The Ascogenous Stage of the Peach Constriction-disease Pathogen.—In PHYTOPATHOLOGY **30**, pages 966 and 967, the writer set forth reasons for considering the species of *Phomopsis* causing peach constriction-disease as a conidial stage of *Diaporthe eres* in the sense of Wehmeyer, or *D. perniciosa* in the sense of certain European investigators, although he had not found an ascogenous stage either occurring naturally or in cultures. Recently perithecia were found in a culture of the peach Phomopsis from Delaware, which, in the above mentioned publication, was called "Peach III." A culture of "Peach III" was transferred to oatmeal agar on Dec. 4, 1940. Five days later a small piece of peach twig, sterilized by flaming, was placed on this vigorously growing culture. The fungus continued to grow vigorously, and in one month produced typical pycnidia on both the agar and the twig. The culture was not again examined until September, 1941, when perithecia were found on both the agar and the twig. These perithecia were

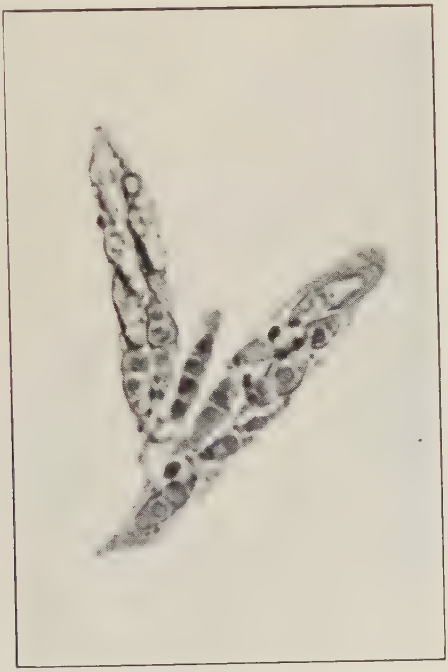

FIG. 1. Asci and ascospores of *Diaporthe* from culture of peach *Phomopsis*. ×950. Mounted and photographed by Marguerite Wilcox.

nearly spherical with short ostioles projecting from the substrate and were typical for the genus *Diaporthe*. Paraphyses were present. The asci were clavate with a refractive ring in the apical wall. The ascospores typically were uniseptate, constricted at the septum, and 4 guttate, although when slightly out of focus they appeared triseptate. Asci measured 49–57 × 8–10 μ; ascospores 12–13 × 4 μ, biseriately arranged (Fig. 1). Attempts to germinate ascospores were unsuccessful, probably because the culture was so old and dry. The morphological characters and measurements of the perithecia, asci, and ascospores support the view that the fungus is *D. eres* in the sense of Wehmeyer or *D. perniciosa* in the sense of many European investigators, and for the present it is so considered by the writer. However, any one working with the various forms of *Phomopsis* occurring on deciduous fruit trees must realize the need for a more thorough investigation of these forms and their ascogenous stages before taxonomic relationships can be determined with certainty.—JOHN W. ROBERTS, Principal Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Beltsville, Maryland.

Poinsettia Scab Discovered in Honolulu.—In December, 1940, E. A. Bessey informed the writer of the discovery of a scabbed condition of a double variety of poinsettia (*Euphorbia pulcherrima* Willd.) in Honolulu, T. H., during his recent stay there. The disease was found in November, 1939, by E. C. Zimmerman, Entomologist of the Bishop Museum. It was discovered in a nursery where, as Dr. Bessey stated, the large number of

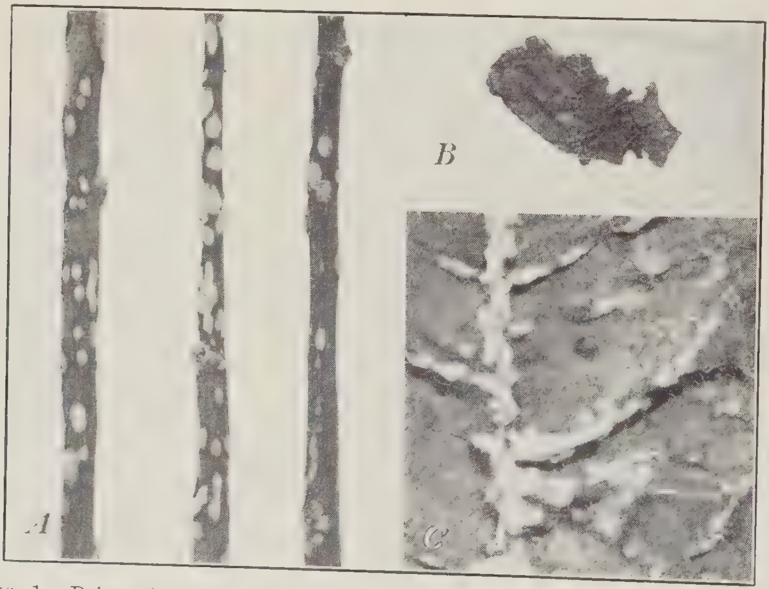

FIG. 1. Poinsettia scab from Honolulu. *A*. Lesion on stems. ×1. *B*. Part of a leaf showing infection principally on midrib and veins. ×1. *C*. Part of *B*. ×8. Photographs by M. L. F. Foubert.

poinsettias grown were much damaged by the attack. From specimens he contributed to the writer early in 1941 (Fig. 1), his preliminary identification of the fungus present as *Sphaceloma* was verified. A pure culture, which he isolated from the scab lesions, as he described it, was probably of the *Sphaceloma*. Dr. Bessey also sent a specimen of the same or a similar disease of *Pedilanthus* sp. This had been collected in a private garden in Honolulu, by the owner, also in November, 1939. In this case further diagnosis awaits additional material. The scab of poinsettia from Honolulu appears to be identical with that found in southern Florida in July, 1940, and subsequently, as recently reported by Ruehle,[1] from whom a specimen is at hand.—ANNA E. JENKINS, Bureau of Plant Industry, Washington, D. C.

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[1] Ruehle, G. D. Poinsettia scab caused by *Sphaceloma*. Phytopath. 31: 947–948. 1941.

REPORT OF THE THIRTY–THIRD ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

THE 1941 DALLAS MEETING

The thirty-third annual meeting of The American Phytopathological Society was held in Dallas, Texas, from December 29, 1941, to January 1, 1942. While the attendance did not equal that of some former years, the meeting was most timely and successful. Seventy-five new members were elected at Dallas, which now gives the Society a membership of 1118.

Approximately 170 attended the Phytopathologists' Dinner at the Hotel Adolphus, and enjoyed the typical southwestern entertainment arranged under the chairmanship of K. Starr Chester, with L. M. Blank and W. H. Tharp assisting.

Special conferences were held on virus diseases from the plant quarantine standpoint, results of cooperative seed-treatment experiments, and plant pathology in relation to national defense and post-war readjustments. The invitation program of demonstrations, under the efficient direction of A. J. Riker, chairman, P. A. Young and E. W. Lyle, again proved its usefulness and was highly commended. The value of cooperation among different branches of plant science was brought out in joint sessions with Section G, of the A.A.A.S., and affiliated botanical societies, the Potato Association of America, and the Mycological Society of America.

The climax of the meeting came with the appointment of a War Emergency Committee, E. C. Stakman, J. G. Leach, and R. P. White, with the power to expand and include members from the five geographical divisions of the country.

The matter of place and date of the 1942 summer meeting will be announced later by the program committee.

OFFICERS, REPRESENTATIVES, AND COMMITTEES FOR 1942

Officers:

L. M. Hutchins, President (1 yr.), Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

J. C. Walker, Vice-President (1 yr.), University of Wisconsin, Madison, Wisconsin.

C. C. Allison, Secretary (3 yrs. term expires 1944), Ohio State University, Columbus, Ohio.

H. A. Edson, Treasurer (3 yrs. term expires 1943), U. S. Department of Agriculture, Washington, D. C.

H. B. Humphrey, Editor in Chief of PHYTOPATHOLOGY (3 yrs. term expires 1943), U. S. Department of Agriculture, Washington, D. C.

Councilors:

J. G. Leach (term expires 1942), West Virginia University, Morgantown, West Virginia.

H. A. Rodenhiser (term expires 1943), U. S. Department of Agriculture, Washington, D. C.

J. G. Horsfall (term expires 1942), Agricultural Experiment Station, New Haven, Connecticut.

F. L. Howard (New England Division), Rhode Island State College, Kingston, Rhode Island.

N. J. Giddings (Pacific Division), P. O. Box 31, Riverside, California.

George M. Armstrong (Southern Division), Clemson Agricultural College, Clemson, South Carolina.

Representatives:

A.A.A.S. Council, Charles Chupp, J. G. Leach.

Group V, Division of Biology and Agriculture, National Research Council, H. P. Barss.

Tropical Research Foundation (5 yrs. term expires 1945), Robert D. Rands.

International Union of Biological Sciences, A. G. Newhall.

Board of Editors, American Journal of Botany (3 yrs. term expires 1943), G. W. Keitt.

Union of American Biological Societies (and Biological Abstracts), H. B. Humphrey and C. C. Allison (*ex officio*), F. V. Rand, C. W. Bennett, H. A. Edson, G. W. Keitt.

Standing Committees:

Donations and Legacies. R. P. WHITE, Chm., E. C. STAKMAN, J. G. BROWN, N. J. GIDDINGS, N. E. STEVENS.

Extension Work and Relations. O. D. BURKE, Chm., R. J. HASKELL, G. W. KEITT, R. H. PORTER, OTTO REINKING, R. C. ROSE, D. R. SANDS, W. B. TISDALE, S. B. FENNE.

Investments. H. A. EDSON, Chm., CHARLES BROOKS, MARVIN E. FOWLER, J. W. ROBERTS.

Necrology. A. G. JOHNSON, Chm., M. B. WAITE.

Phytopathological Classics. H. H. WHETZEL, Manager, H. B. HUMPHREY, Editor.

Public Relations. G. F. WEBER, Chm., J. A. PINCKARD, O. C. BOYD, J. H. JENSEN, FRANK MCWHORTER, A. G. NEWHALL, G. H. STARR, A. J. ULLSTRUP, P. A. YOUNG, C. T. GREGORY.

Regulatory Work and Foreign Plant Diseases. W. A. MCCUBBIN, Chm., A. B. BUCHHOLZ, M. R. HARRIS, S. J. P. CHILTON, A. A. BITANCOURT, F. L. DRAYTON, J. F. ADAMS.

Special Committees:

Coordination in Cereal and Vegetable Seed Treatment Research. M. B. MOORE, Chm., C. H. ARNDT, H. T. COOK, F. J. GREANEY, C. M. HAENSELER, L. D. LEACH, G. L. MCNEW, P. P. PIRONE, H. A. RODENHISER.

Fungus Nomenclature, to Work with British Section of Mycological Society. G. L. ZUNDEL, Chm., C. M. TUCKER, J. A. STEVENSON, D. S. WELCH, ERDMAN WEST.

International Cooperation and Reorganization. L. M. HUTCHINS, Chm., CHARLES CHUPP, W. J. ZAUMEYER, FREEMAN WEISS, J. J. CHRISTENSEN, J. C. WALKER, J. A. STEVENSON, C. W. BENNETT.

Nomenclature and Classification of Plant Viruses. C. W. BENNETT, Chm., JAMES JOHNSON, H. H. MCKINNEY, H. H. THORNBERRY, FREEMAN WEISS, F. O. HOLMES, EUBANKS CARSNER.

Publication of Monographs. MAX GARDNER, Chm., F. D. FROMME, T. F. MANNS, H. S. FAWCETT, L. H. LEONIAN, L. R. HESLER.

Seed Certification. J. C. WALKER, Chm., E. J. WELLHAUSEN, S. J. P. CHILTON, S. M. ZELLER, W. D. MOORE.

Standardization of Fungicidal Tests. S. E. A. MCCALLAN, Chm., J. G. HORSFALL, K. J. KADOW, R. W. LEUKEL, J. W. ROBERTS, C. F. TAYLOR, J. D. WILSON.

Terminology (Nomenclature) of Immunology and Use of Technical Words. NEIL STEVENS, Chm., JESSIE I. WOOD.

War Emergency. E. C. STAKMAN, J. G. LEACH, R. P. WHITE.

Temporary Committees for 1941:

Auditing, R. J. HASKELL, W. W. DIEHL.

Resolutions, O. D. BURKE, G. H. GODFREY, JAMES G. BROWN.

REPORT OF OFFICERS, REPRESENTATIVES, AND COMMITTEES FOR 1941

Report of the Secretary. The society year 1941 opened with 1128 members and closed with 1118, a net loss of 10 members. At the Dallas meeting 75 new members were elected. Six former members were restored to the active roll during the year. The society lost 91 members, 16 by resignation, 4 by death, and 71 by suspension for nonpayment of dues. It might be of interest to the membership to know that, of the 71 suspended, 2 were from South America, 3 from Canada, and 23 from other foreign countries, a total of almost half of those suspended, although the foreign membership is comparatively small. Of the full membership 154 are paid-up life members and two are paying $10.00 a year toward life membership.

The Society's clearing agency, to facilitate contact between employable plant pathologists and pathological openings from individuals or institutions, was continued in 1941. Applications were received or on hand from 55 pathologists. Sixty-six applications from 38 pathologists were sent to 15 prospective employers. It was reported to the agency that three pathologists were hired through contact first made through the agency.

The secretary wishes to thank the members and officers of the Society for their help and cooperation during his four years' service. Special thanks are given to Mrs. O. D. Burke for her painstaking and unselfish secretarial assistance.

Report of the Treasurer. Statement of accounts for the year ending November 30, 1941.

Receipts:

Balance from 1940				$2103.71
Annual dues:				
1939	$ 6.68			
1940	26.99			
1941	2870.60	($10.00, life)		
1942	1958.34	(20.00, life)		
1943	12.50			
1944	10.00			
1945	10.00			
1946	5.00		$4900.11	
Voluntary dues			10.00	
Donations from members for foreign subscriptions			85.00	
Balance from A. P. S. dinner in Philadelphia			20.60	
Index payments included with checks for dues			335.00	
Reimbursement for checks returned by bank			10.00	
Total receipts				5360.71
				$7464.42

Expenditures:

Member subscriptions transferred to PHYTOPATHOLOGY:			
1939	$ 5.68		
1940	20.51		
1941	3667.25	$3693.44	
Transferred to Sinking Fund (Building and Loan)		15.00	
Transferred to PHYTOPATHOLOGY for:			
30-Year Index	278.00		
Donations for foreign subscriptions	85.00		
Voluntary dues	10.00	373.00	
Secretarial work and expenses of office of Secretary		236.58	
Secretarial work for Treasurer		297.50	
Preprints of abstracts		35.46	
Reprints of Constitution		20.88	
Printing		139.44	
Stamps and stamped envelopes		41.03	
Supplies		1.45	
Demonstration programs:			
1940	12.50		
1941	7.41	19.91	
Binding 4 volumes of official set of PHYTOPATHOLOGY		7.60	
Addressing bills		1.11	
Dues refunded		1.50	
Checks returned by bank		10.00	
Collection of checks		1.05	
Total expenditures			4894.95
Balance on hand			2569.47
			$7464.42

Sinking Fund. The Sinking Fund, the income from which is used for the support of PHYTOPATHOLOGY, has been obtained by deducting $5.00 from each life-sustaining membership installment. All but two of the life-sustaining members have paid in full. As one of these is in England and the other in Italy, it will probably be several years before they can complete their payments. The fund totaled $9646.00 at the close of 1940. During the year it increased to $9661.00 and is invested as follows:

First mortgage notes deposited with the McLachlen Banking Corporation for collection ($10000.00 at 6%, $500.00 at 5%)	$1500.00
Invested with the following building and loan associations:	
Arlington and Fairfax Bldg. and Loan, 5%	1000.00
Columbia Permanent Bldg. Association, 4%	510.00
District Bldg. and Loan Association, 4%	1656.08
National Permanent Bldg. Association, 4½%	2000.00
Northwestern Fed. Savings and Loan Association, 3½%	2000.00
Perpetual Bldg. Association, 4%	1020.54
Prudential Bldg. Association, 4% (accrued interest, $2.92)	163.92
	$9850.54
Less interest due PHYTOPATHOLOGY	189.54
	$9661.00

The Lyman Memorial Fund, obtained from voluntary contributions, now totals $3181.53, of which $54.21 represents accrued interest and is available for PHYTOPATHOLOGY. The whole amount is invested with the Brookland Building and Loan Association at 3½%. The account for 1941 follows:

Balance on hand Dec. 15, 1941	$3264.66
Dividends, Dec. 31, 1940, and June 30, 1941	111.16
Contributions from members	20.25
Sales of Erwin F. Smith Memoir	15.00
	3411.07
Accrued dividends, transferred to PHYTOPATHOLOGY, Jan., 1941	229.54
	$3181.53

Report of the Business Manager of PHYTOPATHOLOGY. At the close of 1940 there were 600 nonmember subscribers, including 5 complimentary. During 1941 there were 28 cancellations and 65 suspensions, a loss of 93. This more than offset the 77 new subscriptions and resulted in a net loss of 16, bringing the list at the close of 1941 down to 584, the lowest since 1935. Even this figure will be materially reduced beginning with 1942, when, due to the loss of foreign subscriptions, the list will fall at once to less than 500 with a probability that there will be a further drop of 50 to 100.

Statement of accounts for the year ending November 30, 1941.

Receipts:

Balance from 1940			$ 7288.35
Subscriptions:			
1940	$ 97.10		
1941	2957.19		
1942	381.34		
1943	11.50	$3447.13	
Member subscriptions:			
1939	5.68		
1940	20.51		
1941	3667.25	3693.44	
Sales of back numbers		330.25	
Advertising:			
1940	125.16		
1941	628.74	753.90	
Interest on Sinking Fund:			
First mortgages	85.00		
Building and Loan	454.24	539.24	
Grant from Rockefeller Institute		600.00	
Interest on current funds		173.16	
Interest on Lyman Fund		229.54	
Allowance on reprints		557.70	
From authors for excess illustrations		244.80	
Part payment by National Academy of Science for article in March issue		60.00	
30-Year Index		2040.75	
From A. P. S. for:			
Foreign subscriptions donated by members	85.00		
Voluntary dues	10.00	95.00	
Items for other accounts included with checks for subscriptions and Index:			
E. F. Smith Memoir	1.45		
Classics	3.25		
Lyman Fund	5.25	9.95	
Reimbursement for checks returned by bank		23.75	
Total receipts			12798.61
			$20086.96

Expenditures:

Printing, distributing and storing PHYTOPATHOLOGY:	
Vol. XXX, No. 11	$855.71
No. 12 and Index	798.58

Vol. XXXI, No. 1	895.50			
No. 2	797.99			
No. 3	655.56			
No. 4	761.16			
No. 5	817.03			
No. 6	795.57			
No. 7	808.29			
No. 8	726.11			
No. 9	740.83			
No. 10	721.61	$9373.94		
Postage		580.36	$9954.30	
Secretarial work and office expenses, Editor in Chief			391.91	
Secretarial work, Business Manager			385.60	
Allowance, Advertising Manager			55.88	
Stamps and stamped envelopes			74.72	
Supplies			1.20	
Printing			34.51	
30-Year Index			3171.46	
Interest and storage on paper purchased in advance			62.89	
Transferred to other accounts:				
E. F. Smith Memoir	1.45			
Classics	3.25			
Lyman Fund	5.25		9.95	
Refund, subscriptions and sales			12.66	
Checks returned by bank			23.60	
Bank service charge			4.95	
Total expenditures				$14183.63
Balance on hand:				
Checking account			$1155.17	
Northwestern Fed. Sav's and Loan Association			4748.16	5903.33
				$20086.96

Report of the Auditing Committee for the year ending November 30, 1941. The books of the Treasurer of The American Phytopathological Society and of the Business Manager of PHYTOPATHOLOGY, including accounts of the Society's Sinking Fund and of the Lyman Memorial Fund, have been examined. We have compared receipts and expenditures with bank statements and find them to be complete and satisfactory in every detail.

ROYAL J. HASKELL
WILLIAM W. DIEHL

Dec. 16, 1941.

Report of the Advertising Manager. There were 93 revenue-producing advertisements, occupying 51 pages and consisting of 23 full-page, 42 half-page, and 28 quarter-page insertions. During the year 12 commercial companies used our Journal as advertising medium.

There were 38 nonrevenue-producing advertisements, occupying 24 pages and made up of notices regarding Phytopathological Classics, the Society clearing agency, the Committee on Public Relations, the 30-Year Index and a few others.

Contracts in 1941 totaled $834.26.

AGNES E. MEIER

Report of the Editor in Chief. Volume 31 of PHYTOPATHOLOGY contains, exclusive of the index, 1142 pages of printed matter and illustrations classified as follows: 109 articles, 51 notes, 3 reports of meetings, 7 book reviews, 159 abstracts, 266 text figures, 1 plate, and 2 frontispieces. From December 16, 1940, to December 10, 1941, a total of 195 manuscripts of articles, notes, book reviews and reports of Society meetings were submitted for publication in PHYTOPATHOLOGY. Sixteen of these manuscripts were either recalled by their authors or returned to them as unsuitable for publication in our Journal. There are now in press 46 articles and 103 abstracts. Other manuscripts now on hand (Dec. 10) comprise a total of 754 typewritten pages. It is estimated that there is in hand enough material to fill 770 pages of PHYTOPATHOLOGY. At this time last year the figure stood at 820 pages, a difference of 50 in favor of 1941.

Continued and increased emphasis on the importance, even the necessity, of condensing and otherwise improving manuscripts has made itself felt throughout the past year. There is room for further improvement, and this improvement must be effected by the contributors themselves. There is scarcely a manuscript submitted for publication in our Journal but that will bear more or less condensation without any impairment of quality or scientific worth. Manuscripts are still coming in without table headings and with inadequately described illustrations. Still others are accompanied by illustrations of such poor quality that, when reproduced, they fail to reveal the details called for in the legends or described in the text. It is time and money wasted to submit any but high-quality photographs printed on glossy Velox paper or its equivalent.

No manuscript, carrying citations to literature, should be submitted without assurance of the correctness of all citations. Too many contributors still leave this job to the editor. The value and excellence of our Journal will be no better, no greater, than the papers upon which it depends for its continuance.

H. B. HUMPHREY

Report of the Manager of Phytopathological Classics for the year 1941. I beg to submit herewith the annual report of my stewardship as Manager of Phytopathological Classics:

Report for the fiscal year beginning December 1, 1940,
and ending December 1, 1941

Classics No. 1:	On hand 12–1–40	80	
	Sold during year	15	
	On hand 12–1–41		65
Classics No. 2:	On hand 12–1–40	281	
	Sold during year	14	
	On hand 12–1–41		267
Classics No. 3:	On hand 12–1–40	380	
	Sold during year	18	
	On hand 12–1–41		362
Classics No. 4:	On hand 12–1–40	442	
	Sold during year	17	
	On hand 12–1–41		425
Classics No. 5:	On hand 12–1–40	688	
	Sold during year	16	
	On hand 12–1–41		672
Classics No. 6:	On hand 12–1–40	813	
	Sold during year	20	
	On hand 12–1–41		793

Cash balance on hand 12–1–40	$282.49	
Receipts during year	64.75	
Total		$347.24
Expenditures:		
Postage	$ 2.10	
Total expenditures		2.10
Balance on hand December 1, 1941		$345.14
Due on accounts		$ 8.75

PHYTOPATHOLOGICAL CLASSICS
Number 7

1. CONCERNING THE MOSAIC DISEASE OF TOBACCO
   By Adolph Mayer
2. CONCERNING THE MOSAIC DISEASE OF THE TOBACCO PLANT
   By Dmittri Ivanowskiĭ

3. Concerning a Contagium Vivium Fluidum as a Cause of the Spot-Disease of Tobacco Leaves
By Martinus W. Beijernick
4. On the Etiology of Infectious Variegation
By Erwin Baur

Translated from German
by
James Johnson
With a preface and biographical sketches by the translator
Published by
The American Phytopathological Society
1942

Report of the Necrology Committee. During 1941 there were five deaths within our membership as follows:

Karl von Tubeuf, February 8;
Otis Chester Whipple, February 13;
Lee Ellis Miles, May 10;
Wallace Butler, August 3; and
R. R. McLean, September 28.

A. G. Johnson
M. B. Waite

Summary of the Report of the Committee on Biological Abstracts and the Union of American Biological Societies. *What the Biological Abstracts Staff Is Doing for the Biologists.*—Against great odds and in spite of the war, the staff of *Biological Abstracts* has completed a notable year of accomplishment. Few realize the magnitude of the task performed over the last four years by a central office staff, which had been reduced in numbers to about one-fourth its normal size, nor is the enormous amount of extra time and nervous energy necessarily expended by the few to do the work of the many fully appreciated. Even in face of these handicaps, however, very conspicuous progress has been made.

The war has had a tremendous effect on scientific output and distribution. Now, as never before, the remaining abstract journals are demonstrating their importance. During 1941, therefore, *Biological Abstracts* has made every consistent effort within its power and means to produce abstracts of as much as possible of the literature that the war has made directly unavailable to most biologists outside the countries of origin. In carrying out these endeavors, the aid of many biologists in foreign countries has been obtained in furnishing abstracts from journals not now available here. *Biological Abstracts*, on its side, is also helping British abstract journals to obtain material not now directly available to them.

Toward the ends enumerated, 1550 serial publications are now (December, 1941) being regularly covered as against 1105 early in the year; and 24,759 entries appear in volume 15 as compared with 17,038 for the preceding year. During the first half of 1941 the indexes for 1940 were completed and mailed, a notable feat in itself. Furthermore, all the preliminary correspondence and organization has been completed for the new sectional edition being launched with the first issue of 1942. In the 1941 volume, *Section D—Abstracts of Plant Sciences* carried 7060 entries from about 951 journals, and phytopathology alone had 1215 entries from about 292 journals. In each case the number of entries was approximately doubled and the journal sources were increased by about one-third over the preceding year. Additional journals as pointed to by cross references would add about 30 per cent to the number of journals covered.

What the Biologists Are Doing for Biological Abstracts.—In the giving of time and effort and moral support the biologists have not been found wanting. From past experience your Chairman knows something of what the eleven members of the Board of Trustees and its Secretary have put into the enterprise. There are also 143 specialists on the Editorial Board, and nineteen biologists participate in the indexing. Well over 1000 individuals prepare abstracts of the papers of other biologists, and several hundred journals are now covered through assignments by their editorial staffs for author abstracts. However, as to financial backing, so sorely needed during these war times, scarcely one biologist out of every eight in the United States is said to be making any contribution at all.

With regard to paid subscription commitments, the United States showed a net gain of 31 for the complete and 93 for the sectional editions over the 1940 record. Foreign subscriptions as a whole showed for 1941 a net loss of 11 for the complete and a net gain of 25 for the sectional editions, and this picture would have been much less favorable had it not been for the approximate doubling of subscriptions among 10 Latin American countries. The total subscription commitments (complete and sectional) for 1941 were

2055 for the United States and 834 for all other countries, making a grand total of 2889 and representing net gains of 20 for the complete and 118 for the sectional editions over 1940. To *Section D—Abstracts of Plant Sciences*, there were only 421 subscribers in 1941! With the partial or complete breakdown of most abstracting services outside the United States, we of America owe it to our own selfish interests, as well as to our abstract journals, to give them fuller financial as well as intellectual support.

Outlook for the Future.—Advancement has been made against odds. The hope is that this momentum may be progressively maintained: its continuance rests largely on increasing gains in support by the individual biologists of the country: the greater this financial support the more adequate will coverage of the literature become. An encouraging sign is the increasing awareness by biologists of the United States that *Biological Abstracts* is their own instrument. This is evidenced not only by growing individual interest, but also by a very considerable financial support accorded through some eight national societies with biological proclivities. What then of the future? It is the firm resolution of the responsible staff that year by year *Biological Abstracts* shall be made more complete, prompt, and effective, and in all respects more useful. Judging from the present outlook, the foreign income will be less and the expenses greater during the year ahead. If, therefore, the current prompt and increasingly adequate coverage is to be maintained, the whole-hearted support and cooperation of the biological societies and individual biologists of this hemisphere will be urgently needed, nay indispensable.

At such a time as this our professional group can make an important contribution by increased efforts to insure the maintenance of *Biological Abstracts* at the highest possible level of efficiency. Your committee suggests that each society member do what he can toward this end by helping to augment the number of American subscriptions. The results will be particularly valuable at a time when every resource of science must be made promptly available for National Defense.

FREDERICK V. RAND, Chairman

December 16, 1941.

Report of the Extension Work and Relations Committee. The Extension Work and Relations Committee sponsored one activity in 1941. This was a panel discussion on the subject: ''Plant Pathology in relation to national defense and post-war readjustments'' on December 30 at the Dallas Meeting. A detailed report of this conference will appear in the Extension Pathologist in the near future. At the same session the committee sponsored the showing of kodachrome film depicting the ''Speedsprayer.''

C. C. ALLISON, Chairman, O. D. BURKE, R. J. HASKELL, G. W. KEITT,
R. H. PORTER, OTTO REINKING, R. C. ROSE, D. R. SANDS, W. B. TISDALE.

Report of the Committee for Coordination of Research in Cereal and Vegetable Seed Treatments. The activities of this committee for the past year will be presented under the following subdivisions: vegetables, cotton, cereals, and flax.

Vegetable Seed Treatments. Under the general chairmanship of Harold T. Cook, cooperative seed treatment tests were conducted by thirty-eight cooperators located at thirty-one stations in twenty-five states and in two provinces of Canada. C. M. Haenseler personally supervised the lettuce and sweet corn tests; L. D. Leach, the celery tests; G. L. McNew, the cabbage and beet tests; J. C. Walker—not a member of the committee—the cucumber and pea tests; and Harold T. Cook, the spinach tests.

Summaries of this work will be presented by these supervisors at these meetings, and the detailed results have been mimeographed for distribution to the cooperators and others interested. Although the results of tests in 1940 were mimeographed and distributed at the 1940 meetings, the entire report has since been published in the Extension Pathologist for February, 1941.

It seems desirable to continue the vegetable work, possibly on a somewhat reduced scale, for at least a few more seasons.

Cotton Seed Treatments. The work on cotton seed treatments has been carried on for several years under the auspices of the Cotton Seedling Disease Committee of the Cotton Disease Council and under the direct supervision of C. H. Arndt.

Until January, 1941, when Dr. Arndt was placed on the Cereal and Vegetable Seed Treatment Committee, there was no formal association of the two committee. Since that time, the Cotton Seedling Disease Committee has continued its program, and Dr. Arndt has prepared a special summary of six years of experimental studies of cotton seed treatments. This summary is mimeographed and will be distributed to those interested.

Cereals and Flax. In this field, tests were made to determine the effectiveness of various fungicides in increasing yields of wheat, oats, barley, and flax. Other tests were made to determine more directly the effectiveness of these fungicides in controlling smuts of wheat and oats, and barley stripe. These two series of tests were carried on at nine of the more northern States and three Canadian Provinces.

In addition, a new phase of work dealing with corn seed treatment was undertaken for the first time this year and was placed at six different stations. In these tests, only seedling data were taken, since it is impossible to secure varieties which are adapted in length of the growing season to the various states. While the results this year indicate no very pronounced differences in seedling stand, it is thought desirable to continue this work with possibly some modifications of the present plan. It would be highly desirable to have someone from one of the corn belt states placed on this committee to take full charge of the project.

A complete summary of the results of all these tests for the past three years is being prepared and will be distributed before the next crop season.

At the present time, no work is being done on cereal seed treatments in either the hard red or soft red winter wheat areas or in any of the southern states. It would seem highly desirable that such work should be undertaken; and since the problems and the cooperating stations are distinct from those concerned in the more northern states, it would be desirable to have this undertaken by people located in those areas.

M. B. MOORE, Chairman

Report of the Committee on Standardization of Fungicidal Tests. A joint meeting of your Committee with the American Association of Economic Entomologists' Committee on Insecticide Terminology was held at the time of the Philadelphia convention and a number of problems of mutual interest discussed. The two Committees agreed to cooperate wherever possible in formulating standard methods and terminology. Another joint meeting is planned for the New York Convention in 1942–43.

The preparation of tentative "Standard Methods" or lists of "Standard Terms" has been continued and three more are now available in mimeographed form. In each case these have been prepared by the member or members of the Committee especially qualified in cooperation with other specialists.

The new tentative standards are:

1. Tentative Definitions of Fungicide Terms.
2. Tentative Standardized Laboratory and Greenhouse Procedure for Testing the Relative Fungicidal Efficiency of Chemical Dusts in the Control of Certain Cereal Smuts.
3. Tentative Recommendations on Standard Spray Nomenclature (Part II. Pears and Cherries).

Copies of these may be obtained from the Committee Chairman, Boyce Thompson Institute, Yonkers, New York. Copies are also available for the four methods prepared earlier, namely:

1. Tentative Method on a Standard Bordeaux Mixture for Laboratory Tests and for Determination of Bordeaux Coefficient.
2. Tentative Method for Determination of Mean Particle Diameter of Fungicides.
3. Tentative Speciafications for Slide-Moist Chamber Method of Testing Protective Fungicides.
4. Tentative Recommendations on Standard Spray Nomenclature (Part I. Apples and Peaches).

The Committee again requests that these various methods and terms be subjected to trial and constructive criticisms. Also that suggestions be made regarding new methods and terms suitable for standardization. Your cooperation is especially invited, since, shortly, sufficient time will have elapsed to take action on some of these methods as to (1) adaption by the Society as a Standard Method, (2) modification for further consideration, or (3) discarding.

S. E. A. MCCALLAN, Chairman, K. J. KADOW, J. G. HORSFALL,
R. W. LEUKEL, J. W. ROBERTS, C. F. TAYLOR, J. D. WILSON.

Report of the Advisory Committee for 1941. During the past year a number of proposals and suggestions were submitted to the Advisory Committee on Society Activities and Programs, which were given attention by its members. From the comments received, it was clearly indicated that the programs at present are being conducted in a very satisfactory manner and no general criticism is to be offered.

The following points were considered by the Committee and are suggested to the Council:

1. That a special or standing committee on "Constitutional Revisions and Amendments" be appointed whose duty it would be to initiate matters of this type and weigh suggestions. It is believed that this should not be the function of the Advisory Committee.

2. To avoid triple sessions at the winter meetings wherever possible.

3. Not to feature symposia to the extent of not having any other session at the same time.

4. Since the programs of the Society are so well conducted at the present time, making it difficult to suggest specific improvements it is proposed by an almost unanimous

vote of the Committee that the Advisory Committee on Society Activities and Programs be discontinued, leaving it to future councils to appoint a special or standing committee as the need arises. It has been suggested that there should be a greater turnover of committees and the holding of them together, although inactive for some hypothetical emergency, is unwise because it adds to the machinery with no effect.

Respectfully submitted,

W. J. ZAUMEYER, Chairman, R. H. DRAYTON, A. A. DUNLAP,
J. A. PINCKARD, R. K. VOORHEES, J. C. WALKER, C. E. YARWOOD.

Report of the Committee on Public Relations. During the past year your committee again followed the objectives outlined to the Society at its 1939 meeting (Phytopath. 30: 368). Since this meeting, international events have focused our attention upon increased agricultural production. As a result of these changes, your committee has endeavored to give to the public as much information as possible on the new developments in our science. Every newsworthy article appearing in Volume 31 of PHYTOPATHOLOGY has been submitted to one or more selected news agencies for national distribution.

It is the belief of your committee that even greater efforts should be made to inform the public of the latest developments in plant disease control. That Food for Freedom shall not fail, each member of the Society is personally responsible to the American Public for this information.

For your convenience in fulfilling your responsibilities a list of accredited science writers and their addresses is appended:

Dr. Gobind Behari Lal, International News Service, 235 E. 45th St., New York, N. Y.
Mr. Waldemar Kaempffert, 10th Floor, The New York Times, N. Y.
Mr. William L. Laurence, The New York Times, N. Y.
Mr. Tom Ryland, Time, Rockefeller Center, N. Y.
Mr. Steven M. Spencer, The Evening Bulletin, Philadelphia, Pa.
Dr. Robert Potter, American Weekly, 235 E. 45th Street, N. Y.
Mr. John J. O'Neill, Science Editor, New York Herald Tribune, N. Y.
Mr. Stephen J. McDonough, The Associated Press, Star Bldg., Washington, D. C.
Mr. Howard W. Blakeslee, The Associated Press, 50 Rockefeller Plaza, New York, N. Y.
Mr. Watson Davis, Science Service, 2201 Constitution Ave., Washington, D. C.
Mr. David Dietz, Cleveland Press Bldg., Cleveland, Ohio.
Mr. Gerard Piel, Life, 6 Rockefeller Plaza, New York, N. Y.
Mr. Thomas R. Henry, The Washington Star, Washington, D. C.
Mr. John R. Pfeiffer, Newsweek, RKO Building, Rockefeller Center, New York, N. Y.
Mr. Allen Schoenfeld, Ann Arbor Bureau, Ann Arbor, Mich.
Mr. Albert E. Wiggam, 241 Central Park West, New York, N. Y.
Mr. John White, Washington Times Herald, Washington, D. C.
Mr. Henry Platt, Editor, Predate, 220 East 42nd Street, New York, N. Y.
Miss Harriet Mackintosh, Fairchild Publications, 8 E. 13th St., New York, N. Y.
Mr. Max Gilstrap, The Christian Science Monitor, Boston, Mass.
Special Service Bureau, United Press, Daily News Bldg., New York, N. Y.

Your committee wishes to express special thanks to Mr. Will A. Whitney for contributing freely his time in rewriting numerous scientific articles for the press. The committee also wishes to express its gratitude to the Editor in Chief, the printers, and others for their appreciation of our needs and for the assistance they have rendered.

J. A. PINCKARD, Chairman, O. C. BOYD, C. T. GREGORY,
J. H. JENSEN, FRANK MCWHORTER, A. G. NEWHALL,
G. H. STARR, A. J. ULLSTRUP, G. F. WEBER, P. A. YOUNG

Report of the Committee on Regulatory Work and Foreign Plant Diseases. The conference on ''Virus diseases from the Plant Quarantine Standpoint'' was held in Dallas, Texas, as scheduled on December 29, 1941, with about 60 in attendance.

An introductory paper by Eubanks Carsner called attention to a number of foreign virus diseases worthy of consideration from the quarantine viewpoint, and a second paper by S. A. Rohwer discussed the limitations within which plant quarantines would have to work to give effective exclusion of these virus troubles. He further requested the Society to aid the quarantine authorities in the formulation of a corret national policy toward foreign virus diseases, and to recommend any of these for which quarantine action would be justified.

These papers and subsequent discussion brought out several points worthy of mention.

1. It was emphasized that there is a great lack of knowledge on foreign virus diseases and that studies of these by competent pathologists, especially in their native homes, would be a basic need for intelligent quarantine action.

2. Inspection methods have a very limited usefulness as a quarantine procedure, since the presence of a virus in most incoming plant materials cannot be determined either by

examination or by any practical tests now available. The need for developing virus detection methods was stressed.

3. Various types of quarantine detention for imported plant material likely to carry virus infection were mentioned, including growing the plants here under close observation until health was assured, and the possibility of a more effective isolation, such as could be maintained on an isolated island. Tests by cross inoculation would be a useful feature of this procedure.

4. There would be some usefulness in sending our own plants or their varieties to foreign regions to test their reaction to specific virus diseases occurring abroad, and not yet introduced here.

5. The difficulty of eliminating a virus from living plants by suitable treatment was recognized, and this valuable possibility in quarantine procedure should be energetically explored to add other treatment methods to the few highly suggestive accomplishments already known in this field.

6. Note was taken of the need for a greater number of workers technically trained in virus diseases for service in quarantine problems.

Recommendation

Mr. Rohwer in his address and in the discussion which followed made it clear that the Bureau of Entomology and Plant Quarantine was calling on the Society for its aid in solving the plant quarantine problems involved in foreign virus diseases by expressing its constructive opinion on two points: (1) What should be the correct national quarantine policy toward foreign virus diseases? And (2) recommendations as to any specific virus host, or plant group, or virus complex for which in the interests of this country quarantine action is regarded as justifiable.

Naturally no adequate answer could be made to these questions in the conference itself, and your committee therefore recommends, as a step toward eventual preparation of such answer as may be possible, that this committee be authorized to canvass those members concerned in virus disease activities on the two points raised, with a view to presenting their comment and opinion at a subsequent meeting of the Society for its consideration.

W. A. McCUBBIN, Chairman
M. R. HARRIS
A. A. BITANCOURT

Final Report of the Committee on 30-year Index of Phytopathology. The first half of copy for the 30-Year Index of Phytopathology was taken to the printer on May 9th and the remainder on May 16th, 1941. The published index was consigned to the U. S. Mail for subscribers the latter part of September. The Index Committee therefore thankfully relinquishes its official status (and work!) and entrusts its collective self again to the comparative nirvana of the general Society membership.

As to the index itself, it must now stand or fall on its own metaphorical feet. In a task of this size (30,900 author and subject entries, to say nothing of the much larger number of volume-page references) and in spite of all due care, it seems inevitable that some errors must have crept in. The Chairman would appreciate having any such called to his attention for later inclusion in the customary ''Errata.''

FREDERICK V. RAND, Chairman, H. A. EDSON,
GEORGE L. PELTIER, and N. E. STEVENS

Resolution of Appreciation of Work of F. V. Rand. The Society passed the following resolution and voted that it be published in the report of the 1941 annual meeeting:

> The American Phytopathological Society, assembled in annual meeting at Dallas, Texas, December 29 to 31, 1941, hereby expresses its sincere appreciation to Dr. Frederick V. Rand for his service to the Society in organizing and editing the 30-year Index of our Journal PHYTOPATHOLOGY.
>
> His indefatigable energy and devotion to the task, at great personal sacrifice, have placed the Society and the world at large in debt to him for all time. The achievement is outstanding. No scientific society has issued an analytical index more carefully and critically prepared and edited or more inclusive in its scope and usefulness.
>
> The Society also expresses its appreciation to the members who helped in the preparation of index material for individual volumes under Dr. Rand's direction.

HOWARD P. BARSS, Chairman
E. M. JOHNSON
H. A. EDSON

Resolutions of the Committee on Resolutions. RESOLVED that The American Phytopathological Society express its appreciation to the American Association for the Advancement of Science for the very satisfactory arrangements made by its representatives for the meetings of the several sections of our Society, and particularly for the facilities provided for projection.

RESOLVED that the Society convey to the management and staff of the Hotel Adolphus, and especially to Mr. D. T. Segrest and Miss Harriet Rau, its appreciation for the courteous service extended to our members and for cooperation in providing our officers and committees with unusually commodious quarters for conducting business and scientific programs.

RESOLVED that the Society express to the Committee on Local Arrangements, A. A. Dunlap, chairman, A. L. Harrison, L. M. Hutchins, E. W. Lyle, E. L. LeClerg, G. M. Watkins, and V. H. Young, our gratitude for their fine contributions to the success of the meeting; and to the banquet committee, K. Starr Chester, L. M. Blank, and W. H. Tharp, our appreciation of typical southwestern entertainment.

RESOLVED that the Society commend A. J. Riker, chairman of the demonstrational session, and his aides, P. A. Young and E. W. Lyle, for so efficiently providing facilities for this visual means of presenting scientific facts.

RESOLVED that this Society express its appreciation to R. S. Kirby for his four years of loyal, conscientious, and effective service as the Society's Secretary.

RESOLVED that this Society on behalf of its members express to the officers and members of committees our sincere appreciation for the efforts they have made to promote the interests of the Society throughout the year, and to make this annual meeting instructive and enjoyable.

Respectfully submitted,

O. D. BURKE, Chairman
G. H. GODFREY
JAMES G. BROWN

ACTION BY THE SOCIETY AT THE 1941 DALLAS MEETING

Elections and Appointments. The appointments made, as provided by the Constitution, by the President or the Council since the previous meeting, were approved by the Society in business session. The election committee opened and counted the ballots, and the results were announced to the Society at the banquet. The names of those elected and appointed appear earlier in this report in the list of officers, representatives, and committees. Seventy-five applicants were elected to membership.

The Society confirmed the Council's appointment of the following new members to the Editorial Board of PHYTOPATHOLOGY: George L. Peltier, C. O. Johnston, A. G. Plakidas, and E. S. Schultz.

Reports of Officers, Representatives, and Committees. The reports for the year 1941, as presented on previous pages, were read and accepted.

Revision of Constitution. The Society confirmed the Council's recommendation that Article VII of the Constitution read as follows:

ARTICLE VII

Editors, Committees, and Appointments

> The Editors of the official organ of the Society shall be selected by the Council subject to the approval of the Society.
>
> Temporary, Special, and Standing committees may be appointed at the discretion of the Society.
>
> Standing committees, having revolving membership, shall be appointed by the Council to deal with matters pertaining to Society relationships.
>
> Special committees shall be appointed by the Council to deal with plant pathological problems of general interest to the Society. Each special committee shall be continued for such period as in the judgment of the Council may be needed for the accomplishment of its purpose. A final report shall be made at the termination of this period.
>
> Temporary committees shall be appointed by the President to serve during his administration.

Revision of Standing Rule 9. The Society confirmed the Council's recommendation that Standing Rule 9 read as follows:

> "The following shall be selected by the Council and approved by the Society: two representatives on the Council of the American Association for the Advancement of Science for a two-year term; one representative on the Division of Biology and Agriculture of the National Research Council for a three-year term; one trustee on the Tropical Plant Research Foundation for a five-year term; and, one member of the Editorial Board of the American Journal of Botany for a three-year term."

It was also recommended and confirmed that the subsistence expenses of our representative *will not* be paid at the meeting point. The ticket and subsistence enroute will be paid by National Research Council.

Revision of Standing Rule 5d. It was recommended by the Council and confirmed by the Society that Standing Rule 5d. be deleted to read as follows:

> "d. *Meetings of.* Whenever The American Phytopathological Society meets within the territory of a Division, the Division shall merge its program with that of the parent Society."

Society Affiliation. The Council recommended and the Society confirmed the affiliation of The American Phytopathological Society with the American Society of Agricultural Sciences.

Committee on New Memberships and Subscriptions. At the request of the committee, the Society confirmed the Council's recommendation that the Committee on New Memberships and Subscriptions be discontinued.

Advisory Committee. At the request of the committee, the Council recommended and the Society voted that the Advisory Committee on Society Activities and Programs be discontinued. Appreciation was expressed of the fine work done.

War Emergency Committee. The Council recommended and the Society confirmed the appointment of a special committee on War Emergency, composed of E. C. Stakman, J. G. Leach, and R. P. White, with power to expand the committee, with the understanding that they will add to their membership one man from each of the five regional groups and as many more as in their judgment would be advisable. *Regional representatives* are: Northeast, J. G. Horsfall; Middle Atlantic, R. S. Kirby; Upper Mississippi Valley, I. E. Melhus; South, George M. Armstrong; Pacific Coast, Max Gardner. *Representative of Industry:* J. F. Adams. *Representatives from U. S. Department of Agriculture:* H. P. Barss, W. A. McCubbin, R. J. Haskell. *Canadian Representative:* F. J. Greaney. Ex-officio members: Lee M. Hutchins and C. C. Allison.

Recognition of Merit Committee. The Society confirmed the Council's recommendation that the incoming President appoint a temporary committee to study the matter of some form of recognition for outstanding papers presented before our Society, and that the papers be considered for the A.A.A.S. prize.

Classification of Committees. The Society confirmed the Council's classification of Society committees in accordance with the newly adopted revision of Article VII of the Constitution.

Demonstration Session. The Society confirmed the Council's recommendation that the demonstration session be continued, and that the rules for presentation of regular papers apply to the demonstration session.

Committee expenditures. The Society approved the Council's recommendation that committee expenditures will be honored by the Treasurer only when previously approved by the Council.

Summer Meeting. The Council recommended and the Society voted that the matter of place and date of a summer meeting be decided by the Program Committee.

The 1942 Annual Meeting. The Society confirmed the Council's recommendation that we plan to meet with the A.A.A.S. next year.

ROBERT REDPATH McLEAN

March 7, 1875–September 28, 1941

Mr. McLean was born in Oakland, California. At the age of ten, he moved with his people to Illinois where his father accepted a pastorate. Ten years later, the family returned to California but soon thereafter Mr. McLean returned to Illinois where he was employed in the Marshall Field Department Store in Chicago.

In 1912, Mr. McLean again returned to California, this time to San Diego, where, after a brief connection with the County Horticultural Department, he was employed in the Farm Service Department of the First National Bank. In 1922, he was appointed County Horticultural Commissioner for San Diego County. Later the designation was changed to County Agricultural Commissioner. This position he held to the time of his death.

He was past president of State (California) Association of County Agricultural Commissioners, a member of the board of directors of the Floral Association of San Diego, and also a member of the Rotary Club, Silver Gate Masonic Lodge, and First Baptist Church of San Diego.

Mr. McLean was an honored member of the community, actively interested in all progressive matters. His pleasing personality will be greatly missed.

LEE ELLIS MILES

September 25, 1890–May 10, 1941

Lee Ellis Miles was graduated from Wabash College in 1914, with the degree of Bachelor of Arts, and in 1920 he received the degree of Doctor of Philosophy from the University of Illinois. He spent the summer of 1940 at Harvard University in special mycological studies.

From 1917 to 1920, Dr. Miles held a fellowship in plant physiology at the University of Illinois and during the summers of 1919 and 1920 he was employed by the Bureau of Plant Industry, U. S. Department of Agriculture. From 1920 to 1922, he served as plant pathologist for the Mississippi State Plant Board. From 1922 to 1927, he was Plant Pathologist at the Alabama Agricultural Experiment Station and Professor of Plant Pathology at the Alabama Polytechnic Institute. In 1927–1928, he was Assistant Plant Pathologist at the Washington Agricultural Experiment Station and Assistant Professor of Plant Pathology at the State College of Washington. From 1928 to the time of his death, he served as Plant Pathologist at the Mississippi Agricultural Experiment Station.

While Dr. Miles had rather wide interests in the field of plant pathology, his most active interests in recent years were with the diseases of cotton. He had an active mind, a forceful yet pleasing personality, and a host of friends.

OTIS CHESTER WHIPPLE

February 18, 1907–February 13, 1941

Otis Chester Whipple attended Le Verne College (Los Angeles), Junior College (Sacramento), and the University of California, was graduated from the latter in 1933 with the degree of Bachelor of Science, and received the degree of Doctor of Philosophy from the University of Wisconsin in 1937.

In 1937, Dr. Whipple was appointed to an instructorship in plant pathology at the University of Wisconsin, which position he held to the time of his death.

Dr. Whipple did notable work on diseases of truck crops. He was a young man of unusual promise as an investigator and his pleasing personality won him friends not only among his colleagues but also among growers, canners, and others with whom he came in contact.

ERRATA, VOLUME 31

Page 7, line 6, *read* results mean that *for* results to.

Page 234, paragraph 2, line 7, *read* these *for* there.

Page 238, footnote, 1, *read* J. E. Flynn *for* J. S. Flynn.

Page 357, line 10, *read aurantii for aurantidi.*

Page 423, table 1, *read Microsiphum pisi Kalt. for Microsiphum pisi Kolt.*

Page 470, footnote 3, *read* 1939 *for* 1929.

Page 505, line 2, *transfer* next-to-last line, p. 504, to end of line 2, p. 505, immediately following the last word, to *read* In an old culture on carrots some of . . .

Page 568, table 2, *read granulatus for granulatis.*

Page 627, line 12, *read* reason *for* region.

Page 673, line 14, *read* first leaf appeared *for* first appeared.

Page 935, citation 16, *read* In press *for* Plant Physiol. **16**: 1–18. 1941.

Page 935, citation 17, *read* Willis *for* Wllis.

Page 1045, 5th line from bottom, *read* (*S. holci*) *for* (Sholci).

Page 1076, line 16, *read* 2.5 *for* 25.

OTIS CHESTER WHIPPLE
1907–1941

J. C. WALKER

One of the greatest shocks to a group of scientists is the sudden removal from their ranks by death of a young man on the threshold of a promising and useful career. Such was the case when news was received of the sudden collapse of Otis Chester Whipple at Kenosha, Wisconsin, while driving alone on the highway in connection with his research program in that area. This occurred on February 12, 1941; death followed a few hours later, early on February 13.

Whipple was born at Carrington, North Dakota, on February 18, 1907. He came from a long line of pioneers, one of whom had signed the Declaration of Independence. His parents had moved from Missouri shortly before his birth and when he was six years of age they went to California, where they are still engaged in fruit-growing near Sacramento. As a farm boy, Otis Whipple became thoroughly familiar with the practical aspects of agriculture, an experience that contributed to his pioneering but sound approach to applied plant pathology in later years. After graduation at Sacramento High School in 1926, he attended LaVerne College at Los Angeles for two years. He then returned to farming for another three years, attending Junior College at Sacramento for a year during that interval. He entered the University of California in 1931, where he received the Bachelor of Science degree in 1933.

During a year at Davis, California, under the guidance of Dr. J. B. Kendrick, he developed an interest in plant pathology that continued during his senior year and his first year of graduate study at Berkeley under Dr. M. W. Gardner. In the autumn of 1934 he transferred to the University of Wisconsin for further graduate studies leading to the Doctor's degree, which was granted in June, 1937. He was appointed to an instructorship in plant pathology at the University of Wisconsin in July, 1937, and held that position until his death. During this period he was in charge of the truck-crop-disease summer field laboratory near Kenosha in southeastern Wisconsin. On October 23, 1937, he was united in marriage with Miss Joan Young of Racine, Wisconsin, who survives him.

Whipple was a careful and original investigator. He showed these instincts early in his work with Dr. Gardner on spotted wilt of tomato and other plants. His researches at Wisconsin had to do primarily with diseases of the tomato and carrot, with several virus diseases of peas, beans, carrots, and cucumbers, and with spraying of potatoes, tomatoes, and other truck crops for disease control. At the time of his death he had won his spurs as a mature, independent investigator of unusual promise. He enjoyed the friendly respect and high regard of his colleagues, while outside the laboratory he quickly won the friendship and confidence of growers, canners, and other agriculturists with whom he came in contact.

[VOLUME 32, NUMBER 5, MAY, 1942]

OTIS CHESTER WHIPPLE
(1907–1941)

Publications in which Dr. Whipple was sole or joint author follow:

Spotted wilt of tomatoes and its transmission by thrips. (Abstract) Phytopath. **24**: 1136. 1934.

Spotted wilt of truck crops and ornamental plants. (Abstract) Phytopath. **25**: 17. 1935.

Spotted wilt of garden pea. Phytopath. **26**: 918–920. 1936.

Two strains of cucumber virus on pea and bean. (Abstract) Phytopath. **28**: 22. 1938.

Strains of cucumber mosaic virus pathogenic on bean and pea. Jour. Agr. Res. [U.S.] **62**: 27–60. 1941.

Three years of potato spraying in southeastern Wisconsin. Amer. Potato Jour. **18**: 254–261. 1941.

Injury to tomatoes by lightning. Phytopath. **31**: 1017–1022. 1941.

HETEROTHALLISM AND VARIABILITY IN VENTURIA PIRINA[1]

M. H. LANGFORD AND G. W. KEITT

(Accepted for publication July 28, 1941)

Knowledge of variability in the pathogen is a basic factor in the understanding of infectious diseases and their control. Recent contributions to the knowledge of reproduction in the fungi (*e.g.*, 3, 4, 6, 7, 10, 18, 22) have greatly facilitated genetic studies on phytopathogenic fungi. Demonstrations that new races of pathogenic fungi may arise by hybridization or mutation (24) have increased the knowledge of heritable variation in certain fungous pathogens. While extensive studies of variability and inheritance have been made on the rust and the smut fungi (24) and certain saprophytic Ascomycetes, especially *Neurospora* (*e.g.*, 7, 8, 9, 20), the parasitic Ascomycetes have received less attention. Keitt and Palmiter (17) and Keitt and Langford (16) have recently studied variability and inheritance in the apple (*Malus sylvestris* Mill.) scab pathogen, *Venturia inaequalis* (Cke.) Wint. The present paper reports similar work on the closely related fungus, *Venturia pirina* Aderh., the causal agent of pear (*Pyrus communis* L.) scab.

Wiesmann (26) showed that *Venturia inaequalis* and *V. pirina* are species comprising many biotypes that differ in pathogenicity and in morphologic and physiologic characters. His essential findings with reference to *V. pirina* were confirmed and extended by Herbst (13). Herbst, Rudloff, and Schmidt (14) state that, although valid proof is lacking, it may be assumed that the morphotypes of *V. pirina* and of several other species of *Venturia* are hereditary races in which combinations occur. Keitt and Palmiter (17) have reviewed literature relating to inheritance in *V. inaequalis* and have shown experimentally that combinations are a major source of heritable variations in that fungus.

The work reported herein has dealt with 5 sets of monoascosporic isolates of *V. pirina,* each set comprising 8 isolates from a single ascus, with record of the serial order of the spores. A brief preliminary report of this study has been published (19).

ISOLATION OF THE SETS OF 8 SPORES

In July, 1938, the 8 spores from each of 5 asci were isolated from Anjou pear leaves that had been collected in March near Hood River, Oregon, and forwarded to the writers by Mr. Leroy Childs. These leaves were kept air-dry at 4° C. until the isolations were made. The method of isolation has been reported elsewhere (16).

After all the spores from an ascus had been isolated and had germinated, each was transferred to a separate plate of malt agar. The isolates were numbered in the order of occurrence of spores in the ascus, beginning with the apical one. The sets of 8 spores were designated A, B, C, D, and E in the order of their isolation. Each ascus was taken from a different perithecium.

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CULTURAL CHARACTERS OF THE ISOLATES

Wiesmann (26) and Herbst (13) have shown that monosporic cultures of *Venturia pirina* grown *in vitro* vary greatly in morphologic and physiologic characters. The experiments reported below were performed to study the differences in cultural characters of the 8 isolates from each of several asci and to gain evidence as to the constancy of these characters through several successive monoconidial transfers.

Materials and Methods

The isolates were grown at 16° C. on malt agar (Trommer's malt extract, 2.5 per cent; agar, 1.7 per cent). When the ascosporic colonies were 12 days old, the conidial production, diameter, density, and type of growth of each colony were recorded, and a photomicrograph was taken. Then mycelial transfers and monoconidial transfers were made to malt-agar slants and plates, respectively. Throughout the investigation the mycelial transfers were carried independently of the monoconidial transfers described below.

From each of the 40 ascosporic colonies, 7 monoconidial transfers were made by the method described by Keitt and Langford (16), 3 to plates containing 10 cc. each of clear malt agar and 4 to plates containing 20 cc. After the former had been incubated 12 days, a record similar to that described for the ascosporic colonies was taken. The second group of plates was kept 8 weeks, then photographed. A record was taken of the conidial production, color, type of margin, and diameter of each colony.

Thereafter, the 16 isolates of sets C and E were carried in quadruplicate by monoconidial transfers. At the end of each 8-week period, the cultures were characterized as described above, and monoconidial transfers were made. The isolates were carried in this manner through 8 successive transfers. Photographs were then taken for comparison with those of the ascosporic colonies. All isolates were carried on malt agar slants at 16° C. by mycelial transfers at 8-week intervals.

Results

Differences of Isolates.—The 8 isolates of each set comprised 4 groups of 2 isolates each on the basis of colony characters. The 2 isolates of each pair were identical in colony characters, but distinguishable from all other pairs. Thus, among 40 isolates studied, 20 colony types could be distinguished. Figure 1 illustrates this for sets B, C, and E.

Each group of 2 isolates showing the same colony characters, hereinafter designated pairs of isolates, usually were derived from adjacent spores in the ascus. Thus, figure 1 shows that in sets B and C the pairs are 1–2, 3–4, 5–6, 7–8, while in set E they are 1–2, 3–5, 4–7, 6–8. The occurrence of pairs

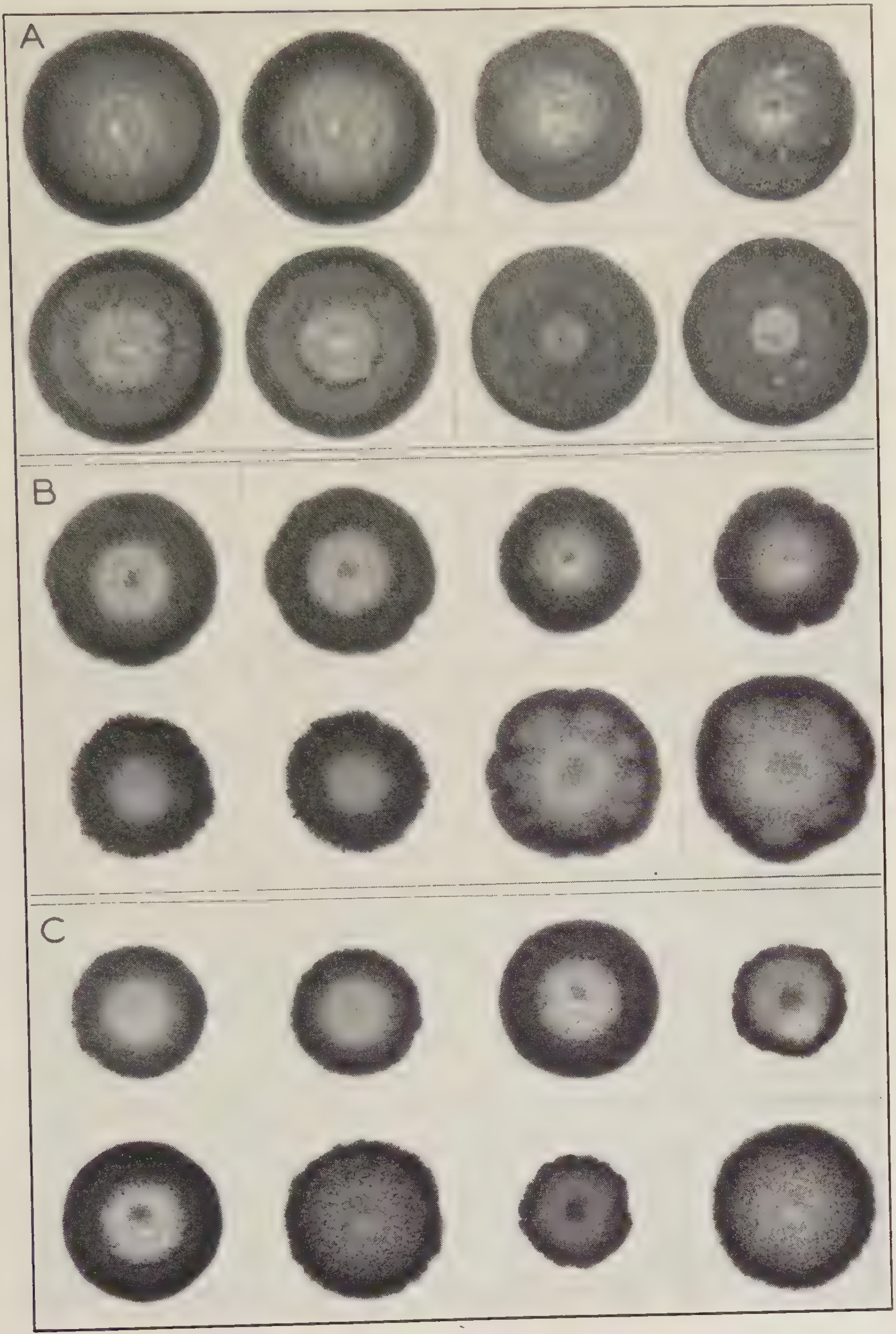

FIG. 1. Monoconidial colonies of *Venturia pirina* after 8 weeks on malt-agar plates at 16° C. The isolates in each set are numbered in the order of occurrence in the ascus of the 8 spores from which they were derived: upper row, left to right, 1, 2, 3, 4; lower row, 5, 6, 7, 8. × ½. A. Set B. B. Set C. C. Set E.

of isolates in set D is the same as that given for set E; in set A it is 1–2, 3–5, 4–6, 7–8.

Assuming that the third nuclear division in the ascus is equational, three possible explanations are offered for the failure in some cases of spores of like genetic constitution to lie next to each other in the ascus: (a) The spindle fibers may be crossed during the third nuclear division. For example, the spindle fibers of nuclei 1 and 2 may overlap those of nuclei 3 and 4, thereby putting nucleus 3 nearer the apex of the ascus than nucleus 2. (b) One nucleus may migrate beyond another before free spore formation begins. (c) Spores may change position. Thus, the arrangement in sets D and E could have resulted from spores (or nuclei) 4 and 5, also, 6 and 7, exchanging places. The arrangement in set A similarly could have resulted from exchange of position of spores 4 and 5.

The nature and extent of differences in colony characters among pairs of isolates within a set varied in the several sets. Thus, the isolates in set B (Fig. 1, A) differed little in growth rate, density, type of margin, or elevation of the central mass, whereas those in set E (Fig. 1, C) differed markedly in all these characters.

The basic color of the aerial mycelium of all isolates studied was brown. The shade of brown, however, varied greatly among the different pairs of isolates. Colonies were often zonate, the center usually being lighter than the outer zones (Fig. 1).

Certain pairs of isolates produced conidia more profusely than others. However, since sporulation was abundant in all cases, no attempt was made to classify the isolates on the basis of conidial production. Also, since the conidia produced by any isolate varied greatly in size, no attempt was made to determine differences in size of conidia produced by different isolates.

Constancy of Cultural Characters.—A comparison of A and B of figure 2 shows that the 8 isolates of set C had undergone little change in microscopic colony characters after passing through 8 successive monoconidial transfers. The macroscopic colony characters showed similar constancy. Set E, barring sectors, remained equally constant in culture. Of the 8 isolates in set C, 232 monoconidial plate cultures were grown for 8 weeks each during the period of 16 months covered by this study. No sectors appeared on any of these. On 220 cultures of set E, grown during the same period and under like conditions, one or more sectors appeared on each of 5 different isolates, a total of 8 sectors having been recorded. Thus, under the conditions of this experiment, the isolates of set E were less stable than those of set C.

Several sectors were isolated and grown under conditions similar to those described for the original isolates. Those that produced no conidia were propagated from hyphal tips. All sector lines had a higher growth rate, as indicated by colony diameter, and produced fewer conidia (none in some cases) than the isolates from which they sprang. They also differed from the original isolates in color, gray sectors frequently arising from brown

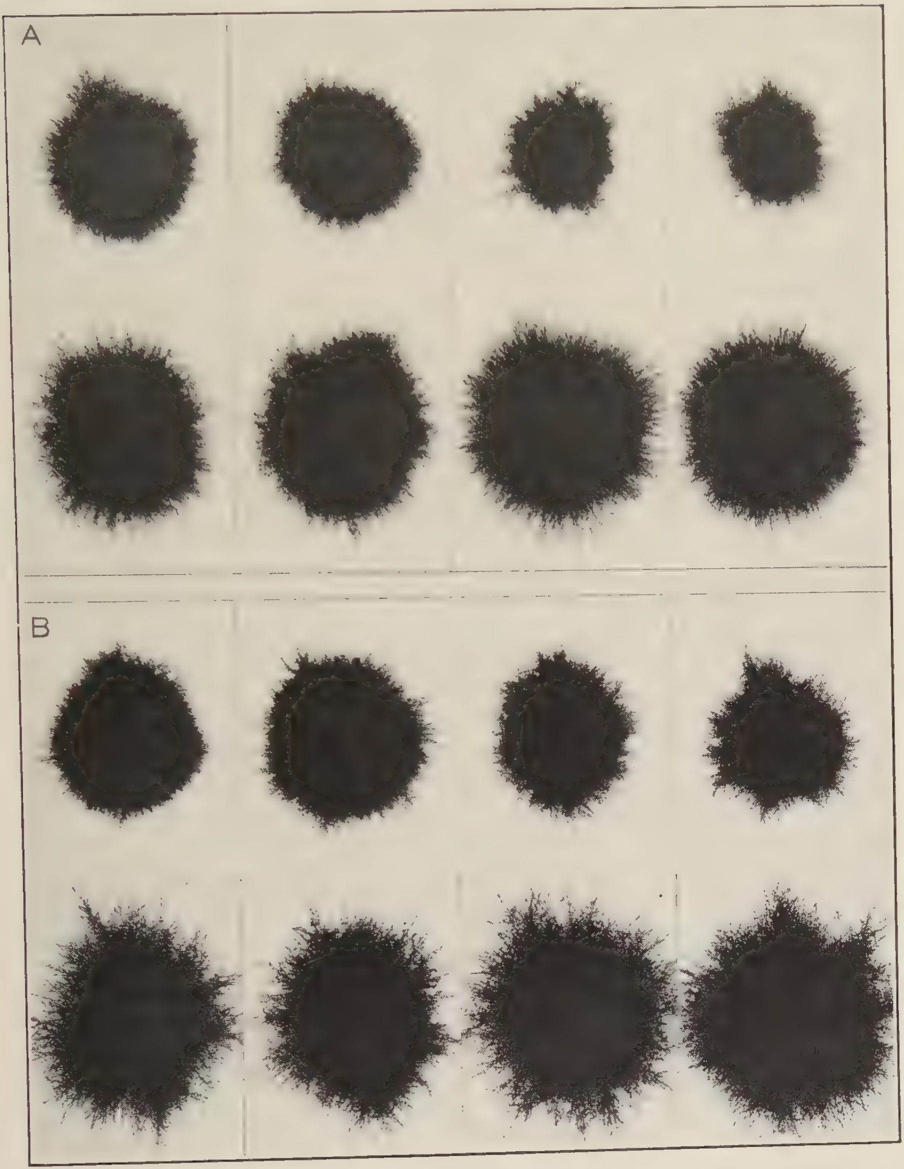

FIG. 2. Photomicrographs of monosporic colonies of the 8 isolates of set C, of *Venturia pirina*, after 12 days on malt-agar plates at 16° C. The isolates in each figure are numbered in the order of occurrence in the ascus of the 8 spores from which they were derived: upper row, left to right, 1, 2, 3, 4; lower row, 5, 6, 7, 8. A. Ascosporic colonies, photographed July, 1938. ×7. B. Colonies of the 8th monoconidial transfer, photographed October, 1939. ×6.

colonies. The colony characters of all sectors have been maintained through several transfers.

BREEDING THE FUNGUS IN VITRO

The writers know of no case in which the production of ascospores of *Venturia pirina* in culture has hitherto been reported. In order to determine whether this fungus is heterothallic or homothallic, a method of producing the ascigerous stage *in vitro* was desired. In 1938, Keitt and Palmiter (17), working with the closely related fungus, *V. inaequalis*, reported that perithecia were produced *in vitro* from some compatible pairings, but not from others. Later, Keitt and Langford (16) developed a method by which mature perithecia of *V. inaequalis* are produced *in vitro* consistently and abundantly. This method, with modifications, was used in producing the perfect stage of *V. pirina* in plate cultures.

Materials and Methods

Preliminary Experiments.—Keitt and Langford (16) found that addition of a decoction of dead apple leaves to malt agar greatly improved it as a medium for growing the ascigerous stage of *V. inaequalis.* Similar use of a decoction of dead pear leaves in the present work gave like results with *V. pirina.* The decoction from leaves of a variety (Kieffer) resistant to the isolates studied was as effective as one from a highly susceptible variety (Anjou), whereas a decoction from McIntosh apple leaves did not stimulate production of perithecia of the pear-scab pathogen. Ash from dead pear leaves showed little or no stimulatory effect. The nature of the stimulatory substance has not been further investigated, but it would appear to be some thermostable organic component of the dead leaves.

Alfalfa stems, sterilized and placed on malt agar before it had solidified, gave a local stimulatory effect, which was, however, less marked than that produced by dead pear leaves. Perithecia were found in some cases partly imbedded in the stems. Pear twigs, used similarly, gave only a slight stimulatory effect, and in no case were perithecia found growing on, or imbedded in, the bark.

The Method Used.—A small piece of mycelium from each isolate was macerated in a separate flask of sterile water. For each pairing about 1 cc. of this suspension of spores and mycelium was poured from each of the 2 desired flasks into a sterile Petri dish. Then 20 cc. of malt agar (Trommer's malt extract, 0.5 per cent; agar, 2.5 per cent), in which a decoction of dead pear leaves had been incorporated, was added to each plate after having been cooled to about 42° C. In order to mix the 2 isolates well, the plate was rotated before the medium had solidified.

The leaf decoction was prepared from dead pear leaves that had been collected in September and stored air-dry in the laboratory. They were placed in a pan, covered with distilled water, and steamed in an Arnold sterilizer for 40 minutes. The decoction from 25 grams of these leaves was incorporated in each liter of medium. The medium was then sterilized at 15 pounds' pressure for 20 minutes.

Using the medium and the technique described above, each of the 40 isolates of the 5 sets was grown separately, and each was paired with every other isolate within its own set of 8. Also, each isolate in set A was paired with each one in set C; and likewise, set B was paired with set D.

Additional pairings were made on dead pear leaves in test tubes. In each tube a leaf was placed so that it adhered to the glass wall and dipped into a 0.5 per cent malt extract decoction, about 3 cc. of which was put into each tube. After sterilization, these leaves were smeared with the fungus. All possible pairings within each of the 5 sets of isolates were made in this manner.

Both the plate and the tube cultures were incubated at 20° C. for 12 days, then at 7° C. until the perithecia came to maturity. The higher temperature was used during the first 12 days to favor vegetative growth without too abundant formation of conidia. The lower temperature checked vegetative growth and favored the formation of perithecia.

Results

Perithecial initials were first observed in some of the cultures 26 days after the experiment was started, or 14 days after the temperature was lowered to 7° C. Ten days later initials were abundant in most cultures. An examination at this time showed that each isolate grown singly had produced both antheridia and ascogonia; thus the fungus is hermaphroditic. Several antheridia were appressed to the trichogyne of each perithecial initial observed. After the cultures had been incubated for 2 months, about half of the pairings had produced abundant perithecia, which were large enough to be visible to the naked eye. Asci were found in some of these. Three weeks later mature ascospores were first observed. An additional month was required to bring the perithecia in most pairings to the peak of maturity, while other pairings produced no mature ascospores until still another month had passed. Thus, some pairings produced ascospores in less than 3 months while others required almost 5 months. Since all plates were seeded at the same time and were grown under like conditions, this difference in time of maturity is thought to be due to a difference in the genetic constitution of the isolates. Attention is invited to the fact that after the first 12 days the plates were incubated at 7° C. It is likely that the time required

TABLE 1.—*Production in vitro of perithecia and ascospores of Venturia pirina from pairings of the 8 monoascosporic isolates of set B*[a]

Isolate number[b]	Isolate number							
	1	2	3	4	5	6	7	8
1	0	0	0	0	+	+	+	+
2		0	0	0	+	+	+	+
3			0	0	+	+	+	+
4				0	+	+	+	+
5					0	0	0	0
6						0	0	0
7							0	0
8								0

[a] 0 = No perithecia, initials only. + = Perithecia containing viable ascospores.

[b] The isolates are numbered in the order of occurrence of the spores in the ascus, beginning with the apical one.

for all pairings to produce mature perithecia would have been shortened by incubating the plates at a higher temperature after the early ascus stage was reached.

Table 1 shows that on the basis of sexual compatibility the 8 isolates of set B fall into 2 groups of 4 isolates each, which are intra-group incompatible and inter-group compatible. Similarly, the isolates of each other set can be divided into 2 groups. All isolates used in this study were found to be self-incompatible.

The results of all possible pairings of the isolates in set B with those in set D are shown in table 2. The 16 isolates of these 2 sets fall into 2 groups of 8 isolates each; isolates B 1, 2, 3, 4, and D 4, 6, 7, 8, composing one group, while B 5, 6, 7, 8, and D 1, 2, 3, 5, compose the other. The isolates of each group are intra-group incompatible and inter-group compatible. Pairings between sets A and C gave similar results. There has been no indication in this work of more than 2 groups for sexual compatibility.

TABLE 2.—*Production in vitro of perithecia and ascospores of Venturia pirina from pairings of the 8 monoascosporic isolates of set B with those of set D*[a]

Isolate number set B[b]	Isolate number, set D[b]							
	1	2	3	5	4	6	7	8
1	+	+	+	+	0	0	0	0
2	+	+	+	+	0	0	0	0
3	+	+	+	+	0	0	0	0
4	+	+	+	+	0	0	0	0
5	0	0	0	0	+	+	+	+
6	0	0	0	0	+	+	+	+
7	0	0	0	0	+	+	+	+
8	0	0	0	0	+	+	+	+

[a] 0 = No perithecia, initials only. + = Perithecia containing viable ascospores.
[b] See footnote b, table 1.

It was mentioned earlier in this paper that some compatible pairings of isolates produced mature perithecia much earlier than others. Figure 3

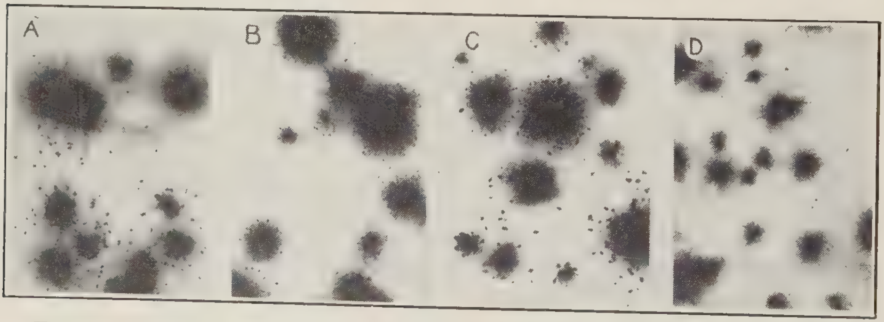

FIG. 3. Perithecia of *Venturia pirina* produced *in vitro* from fertile pairings of isolates, all of which were grown under similar conditions. × 2. A. C 1 × C 3, showing large perithecia. B. C 1 × C 8, showing small perithecia. C. E 3 × E 8, showing abundant perithecia. D. E 7 × E 8, showing sparse perithecia.

shows the variation in number and size of perithecia produced by different compatible pairings. The mature perithecia produced by C 1 × C 3 averaged about 225 μ in diameter, while those produced by C 1 × C 8 averaged no more than 75 μ. E 3 × E 8 produced at least 25 times as many mature perithecia as E 7 × E 8. Furthermore, a single perithecium of the former pairing contained several times as many asci and ascospores as did one of the latter.

The results given above were obtained from plate cultures containing malt agar in which a decoction of dead pear leaves had been incorporated. The cultures on sterilized pear leaves plus malt decoction in test tubes gave similar results. The latter medium, however, is much less satisfactory.

PATHOGENICITY OF THE ISOLATES ON 6 PEAR VARIETIES

In selecting pear varieties for pathogenicity tests with 2 sets of isolates, an attempt was made to use varieties representing different degrees of susceptibility as reported by most investigators (*e.g.*, 1, 2, 5, 11, 21, 23) in this country. Thus, Anjou, Flemish Beauty, Duchess, Seckel, Bartlett, and Kieffer were used. The first 4 varieties are generally listed among the more susceptible ones, while Kieffer is usually classed as highly resistant. Bartlett is considered relatively resistant by most American workers but is classed as highly susceptible in Australia (12, 25).

Materials and Methods

One-year-old pear trees were obtained from a nursery in midwinter. The tops were cut back to 18-inch whips, and the roots were trimmed to fit into 8-inch pots. After being potted the trees were kept in a basement at 10° C. for 12 days, then moved to a greenhouse where the temperature was approximately 16° C. By keeping all buds except 2 rubbed off, 2 vigorous shoots were obtained on each tree. After 8 or 10 leaves had developed on each shoot, the trees were inoculated by atomizing both surfaces of the leaves with a standardized suspension of conidia obtained from cheesecloth "wicks" adherent to the inner wall of 6-ounce bottles, each of which contained about 25 cc. of 2.5 per cent malt extract (Trommer's) solution (17).

In the latter part of March inoculations were made with each of the 16 isolates of sets C and E. One tree of each variety was inoculated with a given isolate. An uninoculated tree was included in each group as a control. The trees were kept in the moist chamber (15) at 16° C. for 48 hours, then returned to the greenhouse, where they were kept 4 weeks before results were taken.

After the results of the first inoculation had been recorded, one heavily infected leaf was picked from each of the Anjou trees, air-dried, and stored in a refrigerator at 4° C. The shoots on all trees were then cut back to about 2 inches and sprayed with Bordeaux mixture. From the buds in the leaf axils, 2 new shoots were allowed to develop on each tree. By June 1 these shoots had 6 to 10 leaves each. The experiment was repeated, inoculating the same trees with the same isolates that were used in the first inoculation.

One shoot on each tree was inoculated with conidia from culture and covered with a glassine bag, while the other shoot was inoculated with conidia of the same isolate from an Anjou leaf. During the day the greenhouse temperature usually rose somewhat above 16° C., but the similarity of the results to those obtained in the first experiment would indicate that this had little effect on disease development.

Results

Table 3 shows the results of inoculations on 6 pear varieties with the isolates of set C. The inoculations in March and those in June gave similar results in practically all cases. In the few instances in which any difference was observed, the higher values are given in the table. The inoculations with conidia from the host (Anjou leaves) and those from culture gave similar results in both number of lesions and type of infection.

TABLE 3.—*Results*[a] *from inoculating pear leaves in the greenhouse with the 8 monoascosporic isolates of Venturia pirina of set C*

Variety	Isolate number[b]							
	1	2	3	4	5	6	7	8
Anjou	++	++	++	++	++	++	++	++
Flemish Beauty	++	++	++	++	++	++	++	++
Duchess	+	+	−	−	+	+	−	−
Seckel	−	−	−	−	−	−	−	−
Bartlett	0	0	0	0	0	0	0	0
Kieffer	0	0	0	0	0	0	0	0

[a] ++ = Abundantly sporulating lesions. + = Moderately or sparsely sporulating lesions. − = Yellowish flecks (no sporulation). 0 = No macroscopic evidence of infection.

[b] See footnote b, table 1.

The differences in varietal susceptibility to the 16 isolates tested are very striking. All isolates incited abundantly sporulating lesions on the leaves of both the Anjou and Flemish Beauty varieties, while none gave any macroscopic evidence of infection on either Bartlett or Kieffer. All isolates incited yellowish flecks but produced no conidia on Seckel leaves, and the 8 isolates of set E gave a similar result on Duchess. The 8 isolates of set C, however, comprised 2 groups of 4 isolates each as regards pathogenicity on the Duchess variety. Isolates 1, 2, 5, and 6 incited sporulating lesions, whereas isolates 3, 4, 7, and 8 gave only flecks. Inoculations with one sector line incited no infection on any variety, while another sector line gave lightly sporulating lesions on Anjou. No lesions or flecks developed on any of the control (uninoculated) trees.

DISCUSSION

On the basis of colony characters, each set of 8 isolates from a single ascus of *Venturia pirina* was found to comprise 4 distinct groups of 2 isolates each. The 2 members of each of these pairs of isolates, which were indistinguishable from each other by any test tried, were derived, with certain exceptions that have been discussed earlier in this paper, from spores that lay next to each other in their order from apex to base of the ascus. It would appear, therefore, that, as in the case of *V. inaequalis* (16), the third nuclear division in the ascus is equational.

The constancy of colony characters and the maintenance of conidial production through 8 successive monoconidial transfers on malt-agar plates would indicate that the method employed has considerable merit for maintaining pure-line cultures. Since a sector of any considerable size can be seen easily on a plate culture, and since most sectors produce conidia sparsely or not at all, this method minimizes the possibility of unwittingly making the transfer from a sector, rather than from the original colony.

The breeding experiments show that *Venturia pirina* is heterothallic, in the sense that the 8 monosporic isolates from each ascus studied comprise 2 groups of 4 isolates each with reference to sexual compatibility, the isolates being hermaphroditic, self-incompatible, intra-group incompatible and inter-group compatible. Evidence from the serial order of the spores in the ascus in relation to the behavior of the isolates indicates that segregation for sexual compatibility may occur alternatively in the first or the second nuclear division in the ascus (*cf.* 16).

The differences among the several isolates in the quantity of conidia produced were not nearly so great as those among fertile pairings in the quantity of ascospores borne. Moreover, under similar conditions, certain pairings produced mature ascospores in 3 months, while others required 5 months. These differences, in both abundance and time of maturity of the ascospores, are thought to be traceable to differences in the genetic constitution of the isolates. It is well known that ascospores may be discharged from leaves in the field over a period of many weeks. Childs (5) reported that in the Hood River Valley of Oregon (the source of the isolates used in this study) ascospores may be discharged from February to September. Although other factors exert an influence, it is suggested that the difference in time required for different pairings of isolates to produce mature perithecia may be an important factor in prolonging the period of ascospore discharge in nature.

No variety of *Pyrus communis* is known to be immune from scab. A survey of the literature, however, shows a lack of agreement among investigators concerning the relative susceptibility of many pear varieties. In some cases, varieties that have been classed as resistant in certain countries or localities have been highly susceptible in others. Doubtless, differences in the environmental conditions and the consequent influence on host susceptibility offer a partial explanation for these discrepancies, but the available evidence indicates that variability in the pathogen is, also, a major factor. Herbst (13) reported the occurrence in some localities of forms of *Venturia pirina* capable of attacking varieties that in other localities remained free of infection. The isolates used in the present study gave only flecking without sporulation on the Seckel variety, which has been reported as highly susceptible by most American investigators (*e.g.*, 2, 11, 21); and no isolate gave any macroscopic evidence of infection on Bartlett, which has been reported as highly susceptible in Australia (12, 25).

The results of the present study afford an experimental demonstration of segregation for pathogenicity in *Venturia pirina.* Isolates 1, 2, 5, and 6 of set C incited typical sporulating lesions on Duchess leaves, whereas 3, 4, 7, and 8 gave only flecks without sporulation. From the serial order of the spores in the ascus in relation to the behavior of these lines it is apparent that in this instance segregation of factors for pathogenicity occurred in the second nuclear division in the ascus.

The facts that the 8 isolates from an ascus comprise 2 groups of 4 isolates each with reference to sexual compatibility and pathogenicity, respectively, and 4 groups of 2 isolates each on the basis of colony characters, justify the conclusion that in *Venturia pirina,* as in *V. inaequalis* (17), both the antheridium and the ascogonium of a fertile pairing contribute hereditary materials to the ascospores, and that segregation of genetic factors precedes ascospore formation. Combinations, therefore, afford an explanation for much of the variation found in this pathogen.

SUMMARY

The 8 spores of each of 5 asci of *Venturia pirina* were isolated in the order of their occurrence in the ascus and grown *in vitro.*

The 40 isolates studied comprise 20 biotypes of 2 isolates each, 4 biotypes being obtained from each ascus. Apparently, the third nuclear division in the ascus is equational.

It was found that the ascigerous stage could be produced abundantly in culture by seeding Petri dishes with conidial suspensions of 2 sexually compatible isolates, adding to each plate 20 cc. of 0.5 per cent malt extract agar in which a decoction of dead pear leaves had been incorporated, and incubating under suitable conditions.

The 8 isolates of each set were grown singly and in all possible pairings of isolates in the same set. The isolates in certain sets were paired with those in other sets in all possible combinations. They were found to be hermaphroditic, but self-incompatible. The 8 isolates from each ascus comprise 2 groups of 4 isolates each, which are intra-group incompatible but intergroup compatible. Likewise, the pairings between sets revealed only 2 groups for sexual compatibility. Segregation of factors for sexual compatibility occurred alternatively in the first or the second nuclear division in the ascus.

Anjou and Flemish Beauty leaves were heavily infected by each of the 16 isolates composing the 2 sets of isolates tested. Kieffer and Bartlett were infected by none of these isolates. Yellowish flecks were incited, but no conidia produced, by all isolates of both sets on Seckel leaves, while all isolates of one set gave similar flecking on Duchess. In the other set, however, 4 isolates incited sporulating lesions on Duchess, while the other 4 incited only flecks.

It is concluded that *Venturia pirina* is a species consisting of many biotypes that differ in morphology and pathogenicity. The fungus is heterothallic, in the sense that the thalli are hermaphroditic but self-incompatible and comprise at least 2 groups that are intra-group incompatible and inter-group compatible. Combinations are, therefore, a major source of heritable variations in this pathogen.

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REDUCTION IN FUNGICIDAL VALUE OF COPPER COMPOUNDS BY ORGANIC MATERIALS

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INTRODUCTION

In modern plant-disease control practices by spraying and dusting, it is more the exception than the rule when a fungicide is used by itself. In orchard spraying a tank-load of spray often contains 4 different materials, while a vegetable dust may contain as many as 5. Each year the list of materials that may be added to fungicides becomes more extensive. These materials, other than insecticides and diluents, are generally classified as wetting, spreading, dispersing, flocculating, buffering, sticking, and ''safening'' agents. Many of these are organic, some being synthetic, while others are pulverized plant products. As the physical and chemical characteristics of these materials differ so widely, a study is being made of their possible action on the fungicidal value of copper compounds.

Several workers have studied certain aspects of the interaction of these materials but scant attention has been paid to their effect on the fungicidal value of copper compounds.

This paper presents the results of a laboratory investigation on the effect of several organic materials on the fungicidal value of copper compounds. While this work was in progress it was learned[3] that J. B. Skaptason and F. M. Blodgett, of Cornell University, had found that derris powder reduced the control of a fixed copper compound against late blight of potato on Long Island. Their work has recently been published (6).

MATERIALS

The materials used in this work were as follows:

Copper Compounds: copper sulphate, copper oxychloride, red cuprous oxide, yellow cuprous oxide, and Bordeaux mixture.

Organic Materials: derris powder (4 per cent rotenone), pyrethrum powder, soya flour, alfalfa meal, corn starch, and activated charcoal (Norite).

Other Materials: Cherokee clay.

METHODS

Considerable attention was paid, for reasons discussed below, to the development of testing methods. Two experimental designs were used in studying the effect of the organic materials: (a) the toxic 50 per cent deposit of copper was held constant and the deposit of organic material varied, and (b) the deposit of organic material was held constant and the deposit of copper varied. In the first design the effect of varying deposits of organic material on fungicidal value at the LD50 point was measured, whereas, in the second design, a measure was obtained of the increase in copper deposit necessary to give LD50 in the presence of unit deposit of organic material. The relative significance of these two designs is being discussed in detail in another paper (1).

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Particular attention to method was not necessary to show that derris, for example, reduced the fungicidal value of a copper compound, but, when the effect of derris on a series of copper materials was studied, particular attention to method was necessary.

It seemed reasonable at first, in accord with most current fungicide research, to compare all the copper materials on the basis of equivalent metallic copper. The fact is, however, that copper spray materials vary widely in their fungicidal value per unit of copper. Apparently, the various materials release different amounts of toxic copper into the spore drop. In view of this, it was necessary to adopt a testing procedure that would permit using the materials on the basis of equivalent *toxic* copper rather than equivalent *metallic* copper, *i.e.,* to so use them that they would give equal spore inhibition. This was done by applying deposits to glass slides which should inhibit 50 per cent of the spores (LD50). By so doing, the effect of the organic materials on the copper compounds was determined at a definite point.

The materials were used in various ways: as sprays, as dusts, or the copper used as a spray and the organic material dusted on the sprayed surface while it was still wet. However, the general testing procedure, except where otherwise noted, was to use the copper materials as sprays and then dust on the organic material. This was found to be the best procedure as the large particle size of some of the organic materials clogged the nozzle of the laboratory sprayer.

The sprayer used was a horizontal precision sprayer (4). The dusting apparatus consisted of a settling tower, a chamber for exposing slides at the base of the tower, and a system for introducing known charges of dust into the tower in uniform time (3). With this apparatus, the deposit of dust can be regulated.

The biological assay method recently described (4) was used to measure the effect of the organic materials on the fungicidal value of the copper compounds. The test fungus was *Macrosporium sarcinaeforme* Cav.

EXPERIMENTATION

As preliminary tests indicated that ground derris root caused reduction in fungicidal value of copper compounds, this material was used as the test organic material.

Effect of Derris on the Same Copper Compound Used as a Spray and as a Dust

Tests were made on the effect of derris on red copper oxide used as a dust and as a spray.

Dust Test. Two dusts were prepared, namely: red copper oxide-Cherokee clay (7–93) and red copper oxide-derris-Cherokee clay (7–18¾–74¼). A series of coated glass slides was dusted with each dust in such a way as to give a series of deposits. The fungicidal value was then determined. The data (Table 1) show that derris reduced the fungicidal value of the copper, that derris by itself had no effect on the test fungus, and that Cherokee clay had no effect on either the copper or the test fungus.

Spray Test. Red cuprous oxide was used alone and combined with derris in the same ratio as used in the dust. Slides were sprayed with each to give a deposit of metallic copper that should inhibit approximately 50 per cent of the spores (LD50). The data (Table 1) show that the derris reduced the fungicidal value of the copper.

TABLE 1.—*Effect of derris on the fungicidal value of a copper compound used as a spray and as a dust*

Material	Metallic Cu deposit	Derris deposit	Spore inhibition
	Gamma/cm.^{2}	*Gamma/cm.*^{2}	*Per cent*
Red cuprous oxide-Cherokee clay (7–93) (dust)	9.1	None	46
	4.5	"	29
	3.1	"	18
	2.6	"	15
Red cuprous oxide-derris-Cherokee clay (7–18¾–74¼) (dust)	7.1	21.4	6
	4.7	18.8	3
	3.5	10.6	1
Red cuprous oxide (spray)	6.0	None	45
Red cuprous oxide-derris (spray)	6.0	18.1	6
Derris (spray)	......	24.0	1
Cherokee clay (spray)[a]	......	....	1
Check		.. .	1

[a] Deposit, 72 gamma/cm.^{2}

The data in table 1 show that derris affects a copper compound alike as a dust or spray.

Effect of Derris on Different Copper Compounds

The tests were made as follows: five slides each were sprayed with Bordeaux and copper oxychloride to give LD50 deposits. One slide for each was held as it was, while the other 4 were dusted while still wet with different charges of derris to give 4 different deposits. The data for a typical test (Table 2) show that equal quantities of derris affected both copper compounds essentially alike at the LD50 point.

TABLE 2.—*Effect of equal quantities of derris on the fungicidal value of Bordeaux mixture and copper oxychloride at the LD50 point*

Copper compound	Derris deposit	Spore inhibition
	Gamma/cm.^{2}	*Per cent*
Bordeaux mixture (metallic Cu deposit, 0.15 gamma/cm.^{2})	None	49
	1.3	51
	2.6	48
	5.1	57
	10.2	31
Copper oxychloride (metallic Cu deposit, 0.30 gamma/cm.^{2})	None	52
	1.3	57
	2.6	50
	5.1	49
	10.2	28
Check	....	2

A test was made also using copper sulphate, a soluble copper compound, in the same maner as described above. The data (Table 3) show that there is a direct correlation between the amount of derris and the reduction in fungicidal value of copper sulphate—the more the derris the greater the reduction.

TABLE 3.—*Effect of amount of derris on the fungicidal value of copper sulphate*

Material	Derris deposit	Spore inhibition
	Gamma/cm.^{2}	*Per cent*
Copper sulphate (metallic Cu deposit, 0.19 gamma/cm.^{2})	None	70
	2.8	55
	5.6	28
	11.2	13
	22.4	0
Check	....	0

From these tests it seems probable that the deleterious effect of powdered derris root on copper fungicides is general as it affected Bordeaux mixture, copper sulphate, copper oxychloride, and cuprous oxide.

Effect of Different Organic Materials on Copper Compounds

In these tests diverse organic materials were used, namely: soya flour (a common supplement); derris powder, pyrethrum powder, and derris extract (insecticides); activated charcoal (a sorbent material); and alfalfa meal (a pulverized plant material not used in sprays or dusts).

Alfalfa Meal, Soya Flour, Derris. The effect of these materials on the fungicidal value of copper oxychloride was determined. The data (Table 4) show that all 3 materials reduced the fungicidal value, soya flour reducing it the most.

Pyrethrum, Derris, and Derris Extract (*Rotenone*). The effect of these

TABLE 4.—*Effect of alfalfa meal, soya flour, and derris on the fungicidal value of copper oxychloride*

Organic material	Deposit of organic material	Copper oxychloride	
		Metallic Cu deposit	Spore inhibition
	Gamma/cm.²	*Gamma/cm.²*	*Per cent*
Alfalfa meal[a]	None	0.32	58
	3.0	"	38
	6.0	"	35
	12.0	"	29
Soya flour[b]	None	0.32	58
	2.1	"	63
	4.2	"	52
	8.4	"	12
Derris[a]	None	0.32	58
	2.6	"	50
	5.2	"	49
	10.4	"	28
Check	....	....	2

[a] Had no effect on the test fungus.
[b] Stimulated germ tube development somewhat.

materials on yellow cuprous oxide was determined. The test was made in a different fashion.

Stock suspensions were prepared of yellow cuprous oxide (1½–100) and of the derris and pyrethrum powders (4–100); stock solutions (1–200, 1–400, and 1–800) of derris extract were prepared also. These stocks represent the concentration at which these materials are used in the field. They were diluted proportionately to strengths at which they could be used in the laboratory. A series of glass slides was so sprayed with each combination as to give a range of deposits; at each deposit the ratio of added material to copper was the same. The data on spore inhibition were plotted on logarithmic probability paper, and the amount of copper deposit necessary to give LD50 for the various combinations was determined (Table 5).

TABLE 5.—*Amount of metallic copper deposit, using yellow cuprous oxide, required for LD50 in the presence and absence of various organic materials*

Material	Metallic Cu deposit required for LD50
	Gamma/cm.²
Yellow cuprous oxide	0.69
Yellow cuprous oxide + derris	1.08
Yellow cuprous oxide + pyrethrum	1.09
Yellow cuprous oxide + derris extract	
(1–800)	0.70
(1–400)	0.65
(1–200)	0.66

The data in table 5 show the following: when either derris or pyrethrum powder was added to yellow cuprous oxide, 57 per cent more metallic copper was necessary to give LD50 than when the copper was used alone; derris extract (rotenone) had no effect on the fungicidal value; the rotenone in the derris powder is not the material responsible for its effect on copper compounds.

Activated Charcoal (Norite). This material was used, as it is highly adsorptive. Its effect on copper oxychloride was determined. Data obtained (Table 6) show that it had no effect on the fungicidal value of copper oxychloride.

TABLE 6.—*Effect of activated charcoal on the fungicidal value of copper oxychloride*

Copper compound	Activated charcoal deposit	Spore inhibition
	Gamma/cm.²	*Per cent*
Copper oxychloride (metallic Cu deposit, 0.50 gamma/cm.²)	None	62
	5.9	63
	15.5	63
Check	....	0

Effect of the Nature of the Organic Material

The data for activated charcoal indicated the reduction in fungicidal value of copper compounds by organic powders probably was not caused by adsorption of soluble copper from solution, while that obtained comparing derris and derris extract (rotenone) indicated that it might be associated with the nature of the organic material. Accordingly, an attempt was made to determine if the nature of the organic material was related to the reduction. Three materials were selected—soya flour (high protein), corn starch (high starch), and activated charcoal (carbon)—and their effect on the fungicidal value of copper oxychloride determined.

Data obtained in a typical test (Table 7) show that the only material to reduce the spore-inhibiting power of copper oxychloride was soya flour,

TABLE 7.—*Effect of nature of the organic material on the fungicidal value of copper oxychloride*

Organic material	Deposit of organic material	Copper oxychloride	
		Metallic Cu deposit	Spore inhibition
	Gamma/cm.²	*Gamma/cm.²*	*Per cent*
Activated charcoal	None	0.41	49
	13.0	0.41	53
Corn starch	None	0.41	49
	13.0	0.41	49
Soya flour	None	0.41	50
	13.0	0.41	33
Check	....	....	1

the material of high protein content. Similar results were obtained also when Bordeaux mixture and red cuprous oxide were used in place of copper oxychloride.

These data suggest the possibility that the reduction in spore inhibiting powers of copper compounds by certain organic materials may be brought about by a reaction between soluble copper and the protein in the organic material.

Various organic materials used in this work were submitted to E. M. Bailey, Chief, Dept. of Analytical Chemistry, Connecticut Agricultural Experiment Station, for protein analysis. The analysis by the Kjeldahl gave for each kind of material the following percentages of protein (N × 6.25): Soya flour, 52.06; alfalfa meal, 13.00; pyrethrum, 11.63; derris, 9.13; corn starch, 0.56.

All of these materials, except corn starch, the material of lowest protein content, reduced the spore inhibiting powers of copper compounds. Further, the spore inhibiting powers of copper oxychloride were reduced the most by soya flour, the material of highest protein content.

DISCUSSION

Although the precise mechanism by which copper inhibits spore germination is not fully understood, it has been somewhat generally accepted that the fungicidal value of copper compounds is related to their solubility—the more soluble the material the higher its fungicidal value. It has been shown by Marsh (5), however, that certain soluble copper compounds, *e.g.,* cupric phthalocyanine, are not toxic; and Goldsworthy and Green (2) have presented evidence showing that the fungicidal value is not directly related to the solubility, but is related to the amount of "ionic" (available) copper produced by the material in solution. Irrespective of the nature of the *toxic* copper the same amount is present at the LD50 point, for example, for all copper materials. Thus, when an added material reduces the fungicidal value it must do so by reducing the amount of *toxic* copper present in solution. This may be done by precipitation of the copper from solution, by adsorption or adsorption of the toxic copper, or by chemical reaction between the toxic copper and the added material.

The deleterious action of organic materials on antiseptics is well known in the field of bacteriology. In relation to fungicides, Goldsworthy and Green (2) have pointed out that organic materials, such as those usually found in ordinary nutrient media, are capable of inactivating considerable quantities of soluble and ionic copper by adsorption.

The fact that activated charcoal did not reduce the fungicidal value of a fixed copper compound indicates that adsorption may not be the explanation for the reduction obtained with the various organic materials in this work.

Data obtained in this investigation indicate that the reduction in fungicidal value of copper compounds by certain organic materials may be brought about by a reaction between the toxic copper and the protein in the organic material. This hypothesis is supported by data showing that materials low in protein did not cause reduction, whereas those having considerable protein did.

The laboratory data presented in this paper on red cuprous oxide are supported by field data secured by Skaptason and Blodgett (6) showing that the addition of derris or pyrethrum, alone or in combination, to this material reduced its control of late blight of potato. However, the use of laboratory data on the effect of organic materials on various copper compounds to forecast what will happen in the field must wait, for reasons discussed below, until field data on several copper compounds are available.

There are two factors involved in the protective value (disease controlling powers) of a copper compound in the field—fungicidal value and tenacity. Unpublished data secured by the writers over a period of years indicate that the protective value of a copper compound is more dependent on tenacity than on fungicidal value. For example, the protective value of red cuprous oxide, a material of high tenacity but comparatively low fungicidal value, is equal to that of copper oxychloride, a material of low tenacity but comparatively high fungicidal value. In the laboratory work reported here the influence of tenacity on the spore-inhibiting powers over a period of time has not been determined. Thus, field tests comparing the effect of organic materials on copper compounds of high fungicidal value and low tenacity, and vice versa, are required to determine the influence of tenacity. Such tests are being made.

Data presented showing that derris extract (rotenone) did not reduce the fungicidal value of a fixed copper compound suggest the possibility that the deleterious effects of derris and pyrethrum powders may be overcome by extracting the insecticidal fractions and impregnating them on a suitable inorganic or non-protein organic diluent for use in dust form.

SUMMARY

A laboratory study has been made of the effect of diverse organic materials on the fungicidal value of copper compounds. The copper compounds used were copper sulphate, Bordeaux mixture, copper oxychloride, and red and yellow cuprous oxide.

Materials containing considerable amounts of protein, *e.g.*, derris powder, pyrethrum powder, soya flour, and alfalfa meal, reduced the fungicidal value. The copper compounds were all affected similarly at a definite point (LD50).

Materials containing little or no protein, *e.g.*, activated charcoal and corn starch, did not reduce fungicidal value.

The reduction in fungicidal value by materials containing protein is

most likely brought about by a reaction between the toxic copper and the protein, thus reducing the amount of toxic copper available to the spores.

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RELATION OF MOISTURE TO INVASION OF TOBACCO LEAVES BY BACTERIUM TABACUM AND BACTERIUM ANGULATUM[1]

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In connection with a study on the method of infection of tobacco leaves by *Bacterium tabacum* Wolf and Foster and by *B. angulatum* Fromme and Murray, previous papers from this laboratory have reported that: (a) tobacco leaves atomized with a suspension of *B. tabacum* become infected only if stomata are open at the time of inoculation (5); (b) there is a correlation between stomatal opening and susceptibility of leaves to water-soaking; leaves can be water-soaked by a stream of water beating against the lower surface only when stomata are open (6); (c) leaves may be injured when kept water-soaked for a period of 24 to 48 hours (13).

The object of the experiments reported in this paper is to determine whether bacteria present in water on the surface of a tobacco leaf can invade the leaf in the absence of water-soaked tissue.

LITERATURE REVIEW

Clayton (3) has reported that water-soaking of tobacco leaves during rainstorms accompanied by wind facilitated invasion by the wildfire organism (*Bacterium tabacum*). Braun and Johnson (1) later found a close relation between the amount of water-soaking and the extent of natural infection by *B. angulatum* in tobacco seed beds.

Clinton and McCormick (4) reported that wildfire infection was not obtained by pouring a suspension of the bacteria onto leaves unless conditions were similar to those necessary for natural infection; but the conditions were not described. Riker (11) concluded that a great many more lesions developed on leaves when plants were placed in a moist chamber before and after inoculation than when placed in the moist chamber only after inoculation. But it has been known for years that wildfire and angular-leaf-spot infection can be produced by forcefully atomizing tender leaves of vigorous plants with a bacterial suspension, without placing the plants in a moist chamber before or after inoculation. The stomata, however, must be open at the time of inoculation, and the atomizer must be held close to the leaf surface; apparently the bacteria are then forced into the leaf through the stomata. Very little, if any, infection occurs if the atomizer is held more than 10 or 12 inches from the leaf surface.

There seems to be no complete agreement regarding the method of invasion of susceptible leaves by pathogenic bacteria. Smith (12) first pointed out the importance of keeping plants moist before spraying them with bacteria to produce stomatal infection. McLean and Lee (10) have shown that citrus canker bacteria can enter leaves through stomata only when the stomatal pores are filled with water. Zaumeyer (14) found that infection of bean leaves by *Bacterium phaseoli* was most severe when plants were kept moist before inoculation. He suggested that this caused substomatal cavities to become well supplied with water, offering the bacteria a free swimming channel from the droplets of moisture on the surface to the interior of the leaf. Leach (9) recently has stated that a film of water connecting the surface of the epidermis with the substomatal chamber appears necessary for stomatal infection by bacteria. Heald (8), on the other hand, visualizes the method of bacterial invasion in the following way: "Suppose a single bacterium of a species pathogenic for the host finds lodgment in a film of water over a stomatal opening. Under favorable conditions cell division follows in rapid succession. The resulting bacteria are pushed or work their way into the stomatal opening and soon come to fill the substomatal chamber."

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[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

MATERIALS AND METHODS

Inoculations were made by pouring a suspension of bacteria onto the lower surface of leaves of vigorously growing Burley tobacco plants in greenhouse and field. Twenty-four-hour beef-peptone-broth cultures of *Bacterium tabacum* and *B. angulatum* were used. Just before inoculation the broth cultures were diluted with 50 parts of sterile tap water. To determine whether the leaves were physiologically susceptible to infection under the tested conditions similar leaves were inoculated by atomizing the bacterial suspension onto the lower surface with a deVilbis No. 15 atomizer, held 2 inches from the leaf, thus forcing bacteria into the leaves.

To water-soak leaves, a stream of water was forced from a 10-cc. B–D Champion hypodermic syringe against the leaf surface. The end of the needle of the syringe was held about 1 inch from the leaf surface. The stream of water was moved as rapidly as tissue became water-soaked. Unless stated otherwise, the stream of water was directed onto the lower surface. The water-soaked condition disappeared within 10 to 30 minutes.

RELATION OF SURFACE MOISTURE TO INFECTION

To determine whether bacteria present on leaf surfaces produce infection if the leaves are kept moist several hours after inoculation, the following experiments were performed.

Stomata Open. Leaves Moist After Inoculation

At 11:00 a.m. May 2, 1939, 4 greenhouse plants were inoculated by pouring a bacterial suspension onto the lower leaf surfaces. Stomata were open. Two of the plants were kept under bell jars 24 hours following inoculation, and the other 2 were kept in the greenhouse atmosphere. Seven days later these 4 plants showed only a few isolated wildfire spots, whereas leaves of 4 similar plants, atomized with the bacterial suspension, were heavily infected. The experiment was repeated 3 times with the same results. Similar results were observed on field-grown plants. In some instances the bacterial suspension was examined microscopically before inoculation, and bacteria were seen to be motile.

Stomata Closed. Leaves Moist Until After Stomata Open

Diachun (5) has reported that tobacco leaves atomized with *Bacterium tabacum* became infected only if the stomata were open at the time of inoculation. The following experiments were conducted to determine whether infection occurs on leaves inoculated when the stomata are closed and then kept moist until the stomata open. The bacteria in the water on the leaf surface then would have access to open stomata. On April 18, 1939, at 8 p.m. 4 small plants were inoculated with the atomizer held 2 inches from the lower leaf surface. Most of the stomata were closed. Two of the plants were placed under bell jars to keep the leaf surfaces moist until the stomata would open the following morning. The other two were left in the greenhouse atmosphere to permit the leaf surfaces to dry. The leaves of the plants under the bell jars were still moist at noon the next day, when the plants were removed from the moist air and placed in the greenhouse. Six days after inoculation only a very few wildfire spots were present. There was no more infection on the leaves that had been kept moist until the stomata opened than on those allowed to become dry. But numerous wildfire spots covered the leaves of similar plants inoculated in the same way at 11 a.m., when the stomata were open. This experiment was repeated 3 times, with the same results. Similar results were obtained in the field.

Leaves Moist Before and After Inoculation

Riker (11) found that tobacco leaves developed a great many more lesions when the plants were placed in a moist chamber before and after inoculation, than when they were placed in a moist chamber only after inoculation. However, under conditions of our test we have not been able to obtain such results. On February 13, 1940, at 11 a.m., 2 potted plants were placed in a moist chamber, where the leaf surfaces became wet and remained covered with moisture. Twenty-four hours later, a bacterial suspension was poured onto the left side of each leaf, both upper and lower surface. Stomata were open. One plant was then returned to the moist chamber for an additional 24-hour period; the other was left in the greenhouse atmosphere. Only a very few wildfire spots developed, usually at an injured place on the leaf. The right side of all leaves was inoculated at the same time as the left side, but the bacterial suspension was atomized onto the lower surface from a distance of 2 inches. Severe infection developed, showing that the leaves were susceptible.

Two similar plants, which had not been kept moist before inoculation, were inoculated at the same time. One was then placed in the moist chamber for 24 hours, and the other kept in the greenhouse. The same degree of infection developed on these plants as on those kept moist before inoculation. This experiment was repeated 5 times with similar results.

It is thought that bacteria present on wet leaves probably fail to gain entrance because of their inability to pass from water on the leaf surface into air spaces in the stomatal pores.

INVASION OF WATER-SOAKED LEAF TISSUES

Clayton (3) stated that ''water-soaked areas facilitated invasion by bacteria but that by far their most important effect was in facilitating the spread of the bacteria through the leaf tissues after infection has occurred.'' He reported that water-soaking must persist for a period of 24 to 48 hours in order to break down resistance of the leaves. Braun and Johnson (1) later reported that in tobacco seed beds a close relation was found between the amount of water-soaking and the extent of natural infection and invasion with *Bacterium angulatum*. In the present report, water-soaked tissues (water-soaked for only a few minutes) are considered in their role as pathways for the bacteria to enter leaves, rather than as prolonged treatment to break down the resistance of tissue or to spread bacteria through tissue of invaded leaves.

To determine whether surface bacteria can invade leaves water-soaked for only a short time, the following tests were made.

Leaves Water-soaked at Inoculation

On July 28, 1939, at 3 p.m., stomata were open. Two interveinal areas of 4 leaves each of a tobacco plant in the field were water-soaked. A suspension of *Bacterium tabacum* was then poured onto the entire lower surface of each leaf. All the water-soaking disappeared within 15 minutes. By August 4 the areas that had been water-soaked and inoculated were dead, dry, and brown, with a yellow halo surrounding the dead area. It is believed that the extensive necrosis was caused by coalescence of the numerous infections resulting from invasion of bacteria through thousands of stomata. (If a dilute suspension of bacteria was used (1–1000) typical scattered isolated wildfire spots resulted.) There was no infection on the parts of the leaves that had been inoculated without being water-soaked (similar to figure 1). Four other leaves were water-soaked but not inoculated. They showed no injury at any time. This experiment was repeated 9 times with similar results.

Like results were obtained with *Bacterium angulatum*. The infection in the water-soaked tissue was, however, not so extensively necrotic as that produced by *B. tabacum*. Infection with *B. angulatum* usually resulted in numerous crowded angular leaf spots but with little coalescence of the spots.

Further tests showed that the results were essentially the same whether the leaves were water-soaked through the lower or upper surface, and whether the bacterial suspension was poured onto the surface through which water-soaking took place or the opposite surface. Stomata are present on both surfaces, and water-soaking apparently floods the entire thickness of the leaf.

Leaves Water-soaked Before Inoculation

If water-soaked tissue was permitted to lose the water-soaked appearance before bacterial suspension was poured onto the leaf surface, the bacteria

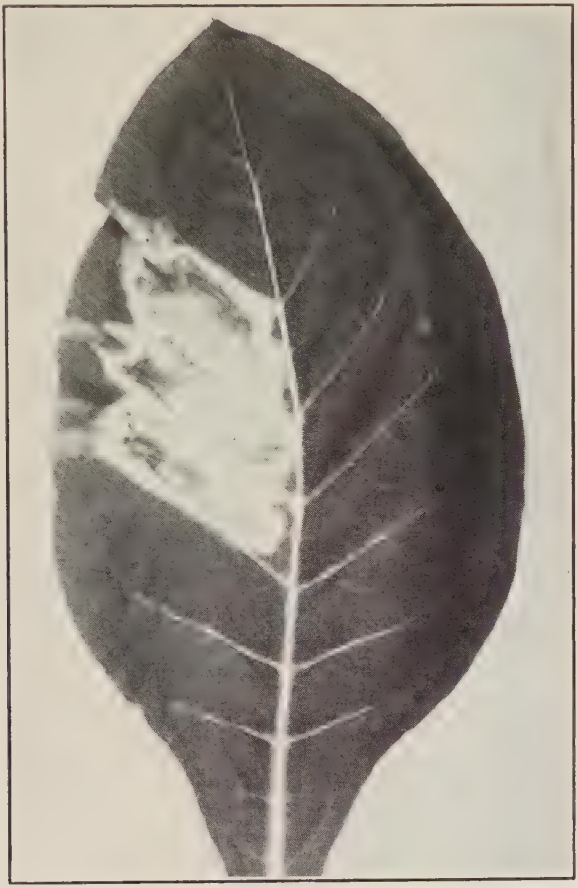

FIG. 1. Invasion of water-soaked areas of a tobacco leaf by *Bacterium tabacum*. The middle three interveinal areas of the right side of the leaf were water-soaked at 10 a.m. September 29, 1939. The water-soaked condition disappeared by 10: 15 a.m. The middle three interveinal areas of the left side were then water-soaked, and a bacterial suspension was poured immediately over the entire lower surface of the leaf. Water-soaking on the left side disappeared within 15 minutes. By October 4 only the areas which were water-soaked at the time of inoculation were dead, brown and dry with a yellow halo. Previous water-soaking had no effect on susceptibility of the leaf to invasion by the bacteria. It is believed that the extensive necrosis was caused by coalescence of infections produced by invasion of thousands of stomata (more than 80,000 per square inch) by the concentrated bacterial suspension. Photographed October 6, 1939.

did not invade the leaf. This is shown by the following test. Three interveinal areas on the left side of a leaf were water-soaked at 2:45 p.m. August 5, 1939. Stomata were open. Water-soaking disappeared by 3 p.m., at which time 2 interveinal areas on the right side of the leaf were water-soaked. Bacterial suspension was immediately poured over the entire lower surface, both left and right sides. Water-soaking on the right side of the leaf disappeared by 3:15 p.m. Infection failed to occur on any part of the leaf except the areas that were water-soaked and inoculated while water-soaking was still visible. By August 9 these were dead, dry, and brown, with a yellow halo. This experiment was repeated 4 times, with the same results (Fig. 1).

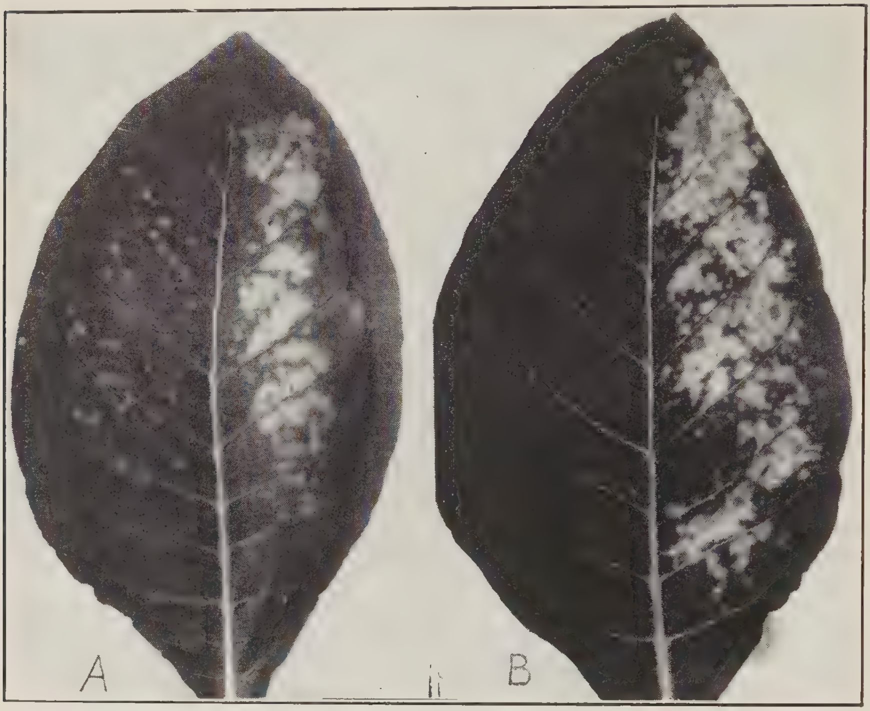

FIG. 2. Bacterial invasion of leaves sprayed with water but not visibly water-soaked. A. Left side atomized with sterile water with atomizer 2 inches from lower surface, and bacterial suspension (*Bacterium tabacum*) immediately poured onto lower surface. B. Bacterial suspension poured onto lower surface of left side without previous spraying with water. Right side of both leaves atomized with bacterial suspension, atomizer 2 inches from lower surface. Inoculated Oct. 9, 1939. Photographed Oct. 17, 1939.

INVASION OF LEAVES SPRAYED WITH WATER BUT NOT VISIBLY WATER-SOAKED

The question naturally arises whether there can be enough free water in the stomatal pores and in leaf tissues to permit bacterial entrance in the absence of visible water-soaking. To answer this question the following test was made. On October 5, 1939, the left side of each of 3 leaves was atomized with sterile water, the atomizer being held 2 inches from the lower surface. There was no visible water-soaking. A suspension of *Bacterium tabacum* was poured onto the lower surface immediately. Within a week there was a considerable number of wildfire spots on these leaves. As controls the left side of each leaf of a second series was inoculated at the same time by pouring bacterial suspension onto the lower surface, without previous spraying with water. No infection developed. The left side of each leaf of a third series was atomized with sterile water, but not inoculated. There was no sign of injury at any time. The right side of each leaf of all 3 series was atomized with bacterial suspension, the atomizer held 2 inches from the lower surface. Severe infection developed on all leaves, showing that all were equally susceptible (Fig. 2). The experiment was repeated 5 times. In 4 cases, the results were similar to these, but in 1 experiment there was total absence of infection on leaves sprayed with water before the bacteria were placed on the surface. The explanation is ventured that, for some reason (probably stomata not open sufficiently), water in sufficient amount was not forced into the stomatal pores and intercellular spaces. The same experiment was repeated 3 times with *Bacterium angulatum*. In each case some infection occurred on leaves sprayed with water (but not visibly water-soaked) before the bacterial suspension was applied.

The results of these tests are interpreted as meaning that when a leaf is sprayed with water, some of the water is driven through the stomata into the substomatal cavities. Not enough water is injected to produce visible flooding or water-soaking, but enough may be injected to form a passage for some of the bacteria subsequently placed on the leaf surface.

DISCUSSION

It is generally believed that in natural field infection *Bacterium tabacum* and *B. angulatum* usually invade tobacco leaves through stomata (2, 4, 7). However, under usual field and greenhouse conditions, bacteria placed on the leaf surface do not produce infection, even though the stomata be open and the leaf surface be kept moist for several hours after inoculation. It appears that the bacteria are unable to pass from water on the leaf surface into the stomatal pores and intercellular spaces. If a continuous passage of liquid exists for a short time from the outer leaf surface, through the stomata, into the intercellular spaces, then bacteria on the leaf surface do gain admission. Temporary natural or artificial water-soaking apparently provides such a liquid passage, for bacteria can infect water-soaked leaves. Natural water-soaking of tobacco leaves is known to occur in plant beds and in the field. In the writers' opinion water-soaked tissues may play an important role as temporary passageways for bacteria to enter leaves, rather than as a prolonged treatment to break down physiological resistance of plants or to spread bacteria within the tissues, as has been suggested by Clayton (3).

Furthermore, experiments show that bacteria, present on the leaf surface, can sometimes invade leaves that are atomized with sterile water just before inoculation, even though the leaves are not visibly water-soaked. This is interpreted to mean that atomizing with water forces some water into the intercellular spaces without actually water-soaking or flooding the tissues. The water on the walls of guard cells and mesophyll cells adjoining the substomatal chambers probably makes a continuous film with the water on the outer surface through the stomata, and permits bacteria to enter. It is not known whether the bacteria gain admission by swimming or whether they are carried in by some physical force.

Nor is it known whether leaf tissues may, under some natural conditions, contain liquid in the intercellular spaces in amount sufficient to serve as a route for bacterial entrance, but not sufficient to produce visible water-soaking. It is conceivable that, under certain natural conditions, water vapor may condense on the surfaces of guard cells and mesophyll cells or that liquid may be secreted internally without producing visible water-soaking. If stomata are open, such liquid could perhaps serve as a passageway for the bacteria to enter leaves, in the absence of visible water-soaking or flooding of tissues.

The authors' present conception of the conditions necessary for bacterial infection of tobacco leaves is as follows: The stomata must be open (or perhaps at times wounds may serve as openings). The bacteria must be driven into the leaf through the stomata by the force of atomizing or of wind-blown rain, or there must be continuous liquid from the outer leaf surface, through the stomata, into the intercellular spaces, to act as a passageway for the bacteria. The excess internal liquid may be visible (water-soaked or flooded tissues), or it may be invisible, and it need exist for only a few minutes. It must be emphasized that in the field these conditions are not satisfied every time leaves are wet, and, therefore, infection does not occur with every rain, even if pathogenic organisms are on the leaves.

SUMMARY

Infection did not develop in the greenhouse or field when a bacterial suspension (*Bacterium tabacum*) was placed on the surface of leaves, even if the leaves were kept moist some time after inoculation; *i.e.*, bacteria did not swim through stomata to produce infection. However, infection did occur when bacterial suspension of *B. tabacum* or *B. angulatum* was placed on the surface of water-soaked leaf tissues. Sustained water-soaking was not necessary. If the inoculum was applied after water-soaked tissues lost the water-soaked condition, infection did not occur. Furthermore, if leaves were sprayed (but not water-soaked) with sterile water prior to inoculation, bacterial suspension then poured onto the sprayed surface produced consid-

erable infection. The writers interpret these results as meaning that before bacterial infection of tobacco leaves can occur stomata must be open, and a liquid passage must exist between the outer leaf surface and the intercellular spaces; or, if this passageway does not exist, the bacteria must be injected forcefully through the stomata.

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RELATION OF PARTICLE SIZE TO FUNGICIDAL VALUE AND TENACITY OF TWO "INSOLUBLE" COPPER FUNGICIDES[1]

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INTRODUCTION

An improvement in the fungicidal value and tenacity of the "insoluble" copper fungicides is to be desired. The latter materials have been developed partly as substitutes for Bordeaux mixture, but have lacked its fungicidal value and tenacity. Copper carbonate and "Tri-Basic" copper sulphate were the two "insoluble" copper materials in which the writer studied the relation of particle size to fungicidal value and tenacity.

MATERIALS

Four samples of copper carbonate and two of "Tri-Basic" copper sulphate were studied. The samples were received as powders, and varied in their fineness.

Physical data concerning the samples are given in table 1. The colors are according to Ridgway (11).

TABLE 1.—*Physical data of the copper compounds used in this study*

Material	Mean particle diameter	Color	Copper	Specific gravity
	μ		*Per cent*	
Dense copper carbonate, A	5.53	Bluish gray-green	56.5	3.86
Dense copper carbonate, B	5.26	Bluish gray-green	56.5	3.86
Light copper carbonate, C	3.13	Pale sulphate-green	56.0	3.68
Light copper carbonate, D	2.68	Pale sulphate-green	56.0	3.68
"Tri-Basic" copper sulphate—coarse	4.07	Turquoise-green	53.5	3.50
"Tri-Basic" copper sulphate—fine	2.54	Turquoise-green	53.5	3.50

METHODS

Technique for Measuring Particle Size

The mean particle diameter, with certain exceptions, was determined by means of the microprojector method used by Heuberger and Horsfall (5). The number of particles per 100 of 20 μ squares was counted directly on the screen and no attempt was made to prove the Poisson distribution of the particles and to use Thorndike's chart. Magnification was 1467 ×. This gave a mean particle size of 2.49 μ for a commercial sample of Yellow Cuprocide (93-per cent cuprous oxide). This is somewhat larger average particle size for this material than Heuberger and Horsfall report (5). However, they used a magnification of 1900 diameters and, by thus including finer particles than were counted in the present study, they would get a smaller mean particle size.

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[1] The writer expresses his thanks to the Tennessee Copper Co., Copperhill, Tenn., for supplying all samples used in this study, to Dr. C. D. Sherbakoff, of the Tennessee Agricultural Experiment Station, for invaluable help in connection with the study, and to Dr. James G. Horsfall, of the Connecticut Agricultural Experiment Station, for examining the manuscript.

All materials were pasted in a small amount of 50-per cent alcohol-water solution and then diluted with distilled water.

Technique for Measuring Fungicidal Value

The fungicidal value was determined according to the tentative specifications of the current committee of The American Phytopathological Society for the slide-moist-chamber method of testing protective fungicides, and on a standard Bordeaux mixture for laboratory tests and for the determination of the Bordeaux coefficient.

The settling tower (10) was employed to apply the materials to the cellulose-nitrate-coated slides. Five deposits per material per test were used. The test fungus was *Macrosporium sarcinaeforme* Cav. The spore concentration was adjusted to 5000 per cc. by means of a blood-counting cell, except for the tests with "Tri-Basic" copper sulphate, in which it was adjusted to 10,000 per cc. The data were plotted on logarithmic-probability paper, whence the LD50 was obtained.

Technique for Measuring Tenacity

The tenacity of the fungicides and a tenacity coefficient were determined according to the method of Heuberger (3).

EXPERIMENTAL DATA

The present paper is concerned only with laboratory studies on the relation of particle size to fungicidal value and tenacity. The data were analyzed by the analysis-of-variance method (12).

Four Copper Carbonates

Fungicidal Value. The Bordeaux coefficient was determined for each of the 4 samples of copper carbonate as received. Four replicates were made on different days. The particle size and Bordeaux coefficients are listed in table 2. The Bordeaux coefficient is derived from the following formula (8):

$$\text{Bordeaux coefficient} = \frac{\text{LD50 deposition of copper in Bordeaux mixture}}{\text{LD50 deposition of toxicant in fungicide X.}}$$

Thus the closer the Bordeaux coefficient approaches unity the closer fungicide X approaches Bordeaux in fungicidal value.

TABLE 2.—*Effect of particle size on fungicidal value and tenacity of four copper carbonates. (Each figure is the mean of four replicates)*

Material	Particle size[a]	Bordeaux coefficient[b]	Tenacity coefficient[c]
	μ		
Copper carbonate, A	5.53	0.047	0.370
Copper carbonate, B	5.26	0.036	0.403
Copper carbonate, C	3.13	0.254	0.492
Copper carbonate, D	2.68	0.276	0.449
Bordeaux mixture	....	....	0.835

[a] The difference in means of A and B is 0.27 μ; of B and C, 2.13; of C and D, 0.45. The least significant difference, at the 5% level, is 0.55 μ.

[b] The difference in means of A and B is 0.011; of B and C, 0.218; of C and D, 0.022. The least significant difference, at the 5% level, is 0.102.

[c] The difference in means of copper carbonates A and B is 0.033; of B and C, 0.089; of C and D, 0.043; of copper carbonate D and Bordeaux mixture, 0.386. The least significant difference, at the 5% level, is 0.094.

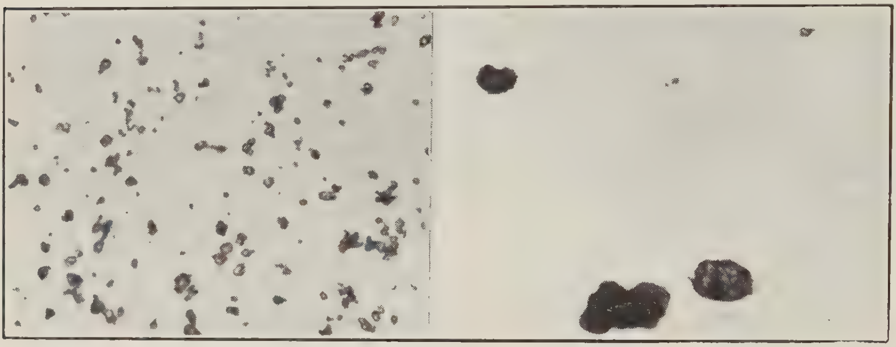

FIG. 1. Photomicrographs of approximately equal weights per unit area of two samples of copper carbonate. Mean particle diameter of samples, left to right, is 2.68 μ; 5.26 μ.

From the data in table 2 we see that an inverse ratio exists between the mean particle diameter and the fungicidal value of the copper carbonates, as measured by the Bordeaux coefficients. That is, as particle size decreases fungicidal value increases. Such relationship has been shown to exist for cuprous oxide (5) and, in the few cases where sound data could be obtained, for a series of "insoluble" copper materials (6).

In this study a significant difference in fungicidal value occurred only when there was a significant difference in the mean particle diameter; that is, between copper carbonates A and B as one group and C and D as another group of smaller particle size. This difference in particle size is vividly illustrated in figure 1. Approximately equal weights of copper carbonate per unit area were obtained for the photomicrographs. This was accomplished by applying equal suspensions of the carbonates to a blood-counting cell and photographing through the cell.

Tenacity. The tenacity of the copper carbonates is seen to increase somewhat as the mean particle size decreases (Table 2). While this difference is not great, hardly being significant, it must be remembered that all samples contained a range of particle sizes, from colloidal on up. The fraction of finer material in samples A and B would tend to minimize the differences in fungicidal value and tenacity between these and samples C and D. This opinion is supported by the data in table 3. Here, the fine fraction of a sample of copper carbonate had greater fungicidal value and tenacity than had the coarse fraction.

Fractionated Copper Carbonate

A sample of copper carbonate was fractionated by elutriation, into definite particle-size classes and their fungicidal value and tenacity determined. This should allow greater precision in the determination of the relation of particle size to fungicidal value and tenacity. The data are presented in table 3.

TABLE 3.—*Effect of particle size on fungicidal value and tenacity of fractionated copper carbonate*

Copper carbonate	Particle size[a] in microns (Mean of 3 replicates)	Bordeaux coefficient[b] (Mean of 4 replicates)	Tenacity coefficient[c] (Mean of 4 replicates)
Coarse fraction	2.06	0.160	0.393
Medium fraction	1.17	0.391	0.512
Fine fraction	0.83	0.534	0.608
Bordeaux mixture	....	....	0.769

[a] The difference in the means of the coarse and medium fractions is 0.89; of the medium and fine fractions, 0.34. The least significant difference, at the 1% level, is 0.27.

[b] The difference in the means of the coarse and medium fractions is 0.231; of the medium and fine fractions, 0.143. The least significant difference, at the 5% level, is 0.138.

[c] The difference in the means of the coarse and medium fractions is 0.119; of the medium and fine fractions, 0.096; of the fine fraction and Bordeaux, 0.161. The least significant difference, at the 5% level, is 0.203.

The fractionation of the copper carbonate was not so complete as desired, but the results show that as the particle size of a given material decreases, its fungicidal value increases. Another important consideration is that, under the conditions of these tests, the tenacity of the fungicide increases as the particle size decreases. If these laboratory studies are a reliable indication of field performance, it would be expected that the protective value of copper carbonate would vary inversely with particle size. This suggestion is exactly supported by the data of Twentyman (13) on field performance of copper carbonate. He found that the finer fractions of a standard copper carbonate gave better control of bunt of wheat than did the coarser fractions.

Heuberger and Horsfall (5) found the protective value of cuprous oxide in the field and in the greenhouse to vary inversely with particle size.

"Tri-Basic" Copper Sulphate

The fungicidal value and tenacity of two samples of "Tri-Basic" copper sulphate were determined. The mean particle diameter of the "fine" sample was 2.54 μ; that of the "coarse" sample was 4.07 μ. The data are presented in table 4.

TABLE 4.—*Effect of particle size on fungicidal value and tenacity of "Tri-Basic" copper sulphate. (Each figure is the mean of three replicates)*

Material	Bordeaux coefficient[a]	Tenacity coefficient[b]
"Tri-Basic" copper sulphate, 4.07 μ particle size	0.181	0.301
"Tri-Basic" copper sulphate, 2.54 μ particle size	0.266	0.507
Bordeaux mixture	....	0.842

[a] The difference in the means of the two samples is 0.085. The least significant difference, at the 5% level, is 0.178.

[b] The difference in the means of the 4.07 μ size and the 2.54 μ size of "Tri-Basic" copper sulphate is 0.206; of the 2.54 μ size and Bordeaux mixture, 0.335. The least significant difference, at the 5% level, is 0.169.

With the "Tri-Basic" copper sulphate, as with the copper carbonates, the fungicidal value and tenacity increased with decreasing particle size. These results show that a further improvement in the insoluble copper fungicides can be achieved by reduction of the particle size.

DISCUSSION

The data presented here show that the fungicidal value and tenacity of copper carbonate and "Tri-Basic" copper sulphate are increased as the mean particle size of the materials is decreased. With copper carbonate this relationship is more clear-cut with the particle size fractions of a given sample than with the several samples of different particle size. This fact emphasizes the desirability of determining the range in particle size of a material as well as its mean particle size in studies of this kind. A narrow range in particle size would be desirable.

The final test of a protective fungicide is its performance in the field. Although in the present study the writer obtained no data on the relation of particle size to protective value, other investigators have done so (5, 13). They found that a decrease in particle size resulted in better protective value. It seems logical that such a relationship would hold true in the present case. One report is to the effect that increasing the fineness of cuprous oxide beyond 325-mesh does not increase its fungicidal value (1). The statement is misleading, however, because 325-mesh measures neither fineness nor coarseness; it merely separates a material into two fractions. One fraction will pass through the screen, the other will not.

SUMMARY

A laboratory study was made of the relationship of particle size to fungicidal value and tenacity of copper carbonate and "Tri-Basic" copper sulphate.

An inverse relationship was found between the particle size and the fungicidal value and tenacity of both insoluble copper materials; that is, fungicidal value and tenacity increased as the size of the particles decreased.

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TEMPERATURE AS IT AFFECTS SPORE GERMINATION IN THE PRESENCE OF COPPER AND SULPHUR

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The effect of temperature on toxicity of fungicides has been studied to a limited extent only. The general opinion has been that as the temperature increases the toxicity of a fungicide increases and that depression of temperature begets a corresponding reduction in toxicity. The optimum temperature for the growth of the organism involved was not considered. Clark (2), however, stated that the temperature at which a toxic material is applied is very important. In a number of experiments in which the toxicity of mercuric chloride to *Aspergillus* was tested, he concluded as follows: "In general, then, mercuric chloride may be said to be more effective either above or below the optimum temperature for the development of the organism or organisms to be checked; . . . This is probably true of all antiseptics which, like mercuric chloride, check or destroy by precipitation of the contents of the living cell. It *may* be true of all toxic agents." No data were presented to substantiate these statements. Brooks (1), studied the relation of temperature and toxic action of sulphuric acid, nitric acid, and copper sulphate solutions in sugar-beet decoction to spore germination and growth of *Botrytis vulgaris, Monilia fructigena, Sterigmatocystis nigra, Mucor mucedo,* and *Penicillium glaucum,* and arrived at the same conclusion. Doran (3) found some dilutions of copper fungicides were toxic when the temperature was near the minimum or maximum for the germination of the spores he employed and the same dilutions were not toxic at the optimum temperatures for the germination of these spores. He states: "We cannot consider a fungicide toxic unless it is toxic under optimum conditions for spore germination." The data presented in this paper in the form of graphs show that copper sulphate and a particulate sulphur inhibit spore germination least at temperatures which are optima for the germination of spores of the organisms concerned.

METHODS

Conidia of *Sclerotinia fructicola* (Wint.) Rehm, *Alternaria solani* (E. and M.) Jones and Grout, *Venturia inaequalis* (Cke.) Wint., and *Sphaerotheca pannosa* (Wallr.) Lév. var. *rosae* Wor., were used in this study as well as urediospores of *Uromyces caryophyllinus* (Schr.) Wint. Spores of *Sclerotinia fructicola* and of *Alternaria solani* were obtained from cultures that had been growing on potato-dextrose agar for 8 to 12 days. Those of *Venturia inaequalis* were from cultures approximately 2 weeks old produced on cheesecloth wicks by the method of Palmiter (6). Urediospores of *Uromyces caryophyllinus* were obtained from Joan Marie carnation plants, which were naturally infected in the greenhouse. Conidia of *Sphaerotheca* *pannosa* var. *rosae* were obtained from actively sporulating lesions on greenhouse roses.

For each organism, except *Sphaerotheca pannosa* var. *rosae,* the spores were germinated in distilled water on glass slides in moist chambers. The spore concentration was adjusted to 50,000 per cc. For each temperature employed, 2 drops of the spore suspension were placed on each of 3 sprayed slides and on each of 3 unsprayed slides. Thus, in each repetition of an experiment, spore germination was observed on 6 slides at each temperature. The 3 unsprayed slides served as a check on germination at each of the temperatures. The percentage germination in all cases was determined by counting 100 spores per slide after 24 hours. Temperatures at 3-degree intervals from 6 to 33° C. were used.

When conidia of *Sphaerotheca pannosa* var. *rosae* were employed, portions of young rose leaflets were used as the test surface, since erratic germination occurs on glass slides (4). The number of spores germinating in each 100 counted was determined after 24 hours by observing the spores *in situ* with the aid of a Leitz Ultrapak microscope. Leaflets were cut along the midrib and one half was sprayed by means of the settling tower and the other half left unsprayed as a check. Conidia were dusted over the sprayed and unsprayed half-leaflets. The temperatures employed were 9, 15, 18, 22 and 28° C. For every repetition of a given experiment 3 unsprayed and 3 sprayed half-leaflets were used at each temperature.

The toxicants employed were a proprietary particulate sulphur[1] and a C.P. grade of copper sulphate ($CuSO_4 \cdot 5H_2O$). For a given experiment the toxicant was applied simultaneously to all of the glass slides (or half-leaflets) to be treated, by means of a settling tower similar to the one described by McCallan and Wilcoxon (5). This assured an equal spray deposit on all of the slides in any one experiment. The spray deposit was regulated so as to allow a spore germination of slightly above 50 per cent on the slides at the optimum temperature for spore germination. When the glass slides were used each experiment was repeated 3 or 4 times; when rose leaflets were used the experiment was repeated 8 times.

EXPERIMENTAL RESULTS

Conidia of *Sclerotinia fructicola, Venturia inaequalis,* and *Sphaerotheca pannosa* var. *rosae* were used in studying the effect of temperature on the toxicity of sulphur. The percentage germination of the checks at the various temperatures is shown in figure 1, A. The optimum temperature for the germination of the conidia of the 3 organisms was approximately the same, 21° C. In figure 1, B, the germination ratio obtained by dividing the average percentage germination on the treated surfaces by the average percentage germination on the check surfaces at each respective temperature is represented. Thus, the higher the ratio the less effective the toxic agent in inhibiting spore germination, and the lower the ratio, the more effective the toxic agent. The data show that the highest ratios occur at approximately the optimum temperatures for spore germination for the three organisms used.

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[1] Walcolized Sulphur obtained from Walco Products, Inc., 512 Greenwich St., New York, N. Y.

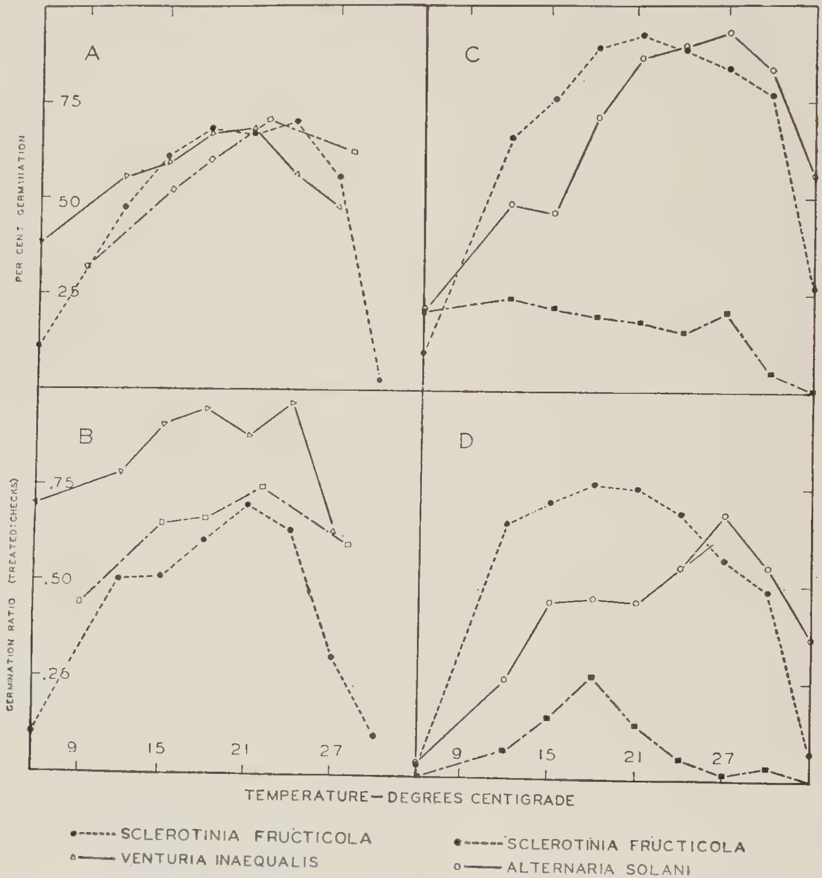

FIG. 1. Graphs showing the percentages of spores germinating on unsprayed glass slides and rose leaflets (A and C) at different temperatures and the ratios of spore germination on sprayed and unsprayed glass and leaf surfaces at the same temperatures (B and D). A. Percentage germination of conidia of *Sclerotinia fructicola* and *Venturia inaequalis* on unsprayed glass slides and of *Sphaerotheca pannosa* var. *rosae* on unsprayed rose leaflets. B. Ratios of the corresponding spore germination on glass slides and on rose leaflets as affected by a particulate sulphur. C. Percentage germination of conidia of *Sclerotinia fructicola*, *Alternaria solani*, and of urediospores of *Uromyces caryophyllinus* on unsprayed glass slides. D. Ratio of the corresponding spore germination on glass slides as affected by copper sulphate.

In studying the effect of temperature in relation to the effect of copper on spore germination, conidia of *Alternaria solani*, *Sclerotinia fructicola*, and urediospores of *Uromyces caryophyllinus* were used. The percentage germination of the checks in the respective temperature chambers is graphically illustrated in figure 1, C. It should be noted that the optimum temperature for *Sclerotinia fructicola* is 21° C., while that for *Alternaria solani* is 27. The germination obtained in these experiments with *Uromyces caryophyllinus* was low and cannot be considered conclusive. It merely offers indicative evidence. The ratios are graphically represented in figure 1, D. The maximum ratios (where copper inhibited spore germination least) occur at temperatures of 18, 18 21, and 27° C. for *Uromyces caryophyllinus*, *Sclerotinia fructicola*, and *Alternaria solani*, respectively.

DISCUSSION AND CONCLUSIONS

The data presented accord with the conclusions of Clark (2) and of Brooks (1), namely, that toxic materials are least effective at the optimum temperature for the organism concerned and that both above and below this optimum temperature these materials are more toxic.

There are many practical implications involved in the question of the relation of temperature to toxicity. Knowledge of this relation may aid the pathologist in recommending spray programs or may help him to explain why a particular fungicide has given excellent results one season but not the next. It may be of considerable importance to the greenhouse man, since, during the winter, he can temporarily adjust the temperature to meet the recommendation. It would seem from these data that in order to destroy a particular pathogen, it would be better to apply a toxic material when the temperature is above or below the optimum temperature for this organism. However, the time required for the killing of an organism by a toxic agent at temperatures below the optimum for that organism would probably be longer than that required at higher temperatures. Therefore, in most cases it would be more desirable to apply a toxic agent when the temperature is above the optimum. An example of a case in which sulphur was more effective at a low temperature than at the optimum was reported by Smith (7). He stated that dusting sulphur was effective in controlling brown rot of peaches under storage conditions at temperatures of 40, 45, and 55° F. (4.5, 7.0, and 13° C.), but that no control was obtained in the lots at temperatures of 65 to 85° F. (18.5 to 29.5° C.). The peaches to which sulphur was not added were readily rotted at all temperatures. If these temperatures are compared with the optimum for *Sclerotinia fructicola* in figure 1, A, or 1, C, it will be seen that the temperatures where no control was obtained were in the range of the optimum for the fungus, whereas those temperatures where control was obtained were much below the optimum.

The data presented in this paper, however, deal only with spore germination. Further studies need to be made of the relationship of temperature and fungicides to the control of plant diseases.

SUMMARY

Studies have been made on the effect of temperature on the fungicidal activity of copper sulphate and a particulate sulphur at temperatures be-

tween 6 and 33° C., inclusive. Copper sulphate was employed against conidia of *Sclerotinia fructicola* and *Alternaria solani* and against urediospores of *Uromyces caryophyllinus*. Sulphur was employed against conidia of *Sclerotinia fructicola*, *Venturia inaequalis*, and *Sphaerotheca pannosa* var. *rosae*.

Copper sulphate and sulphur were least effective at the optimum spore germination temperatures of the organisms employed.

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SOME FACTORS AFFECTING THE INFECTION OF TOMATO SEEDLINGS BY ALTERNARIA SOLANI[1]

W. D. Moore

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INTRODUCTION

During recent years the southern production of vegetable seedlings for transplanting in the North, has developed into an important agricultural industry. Of the several crops grown, the tomato is of greatest importance both in value and in acreage planted. With these field-grown plants, the leaf spot and stem canker caused by *Alternaria solani* (E. and M.) J. and G. has been one of the most important of tomato disease problems. Tomato seedlings grown in the South during the early spring are produced under conditions different from those encountered by plants grown under protection in the North, since they are subject to variable weather conditions and undergo considerable handling during harvesting and packing for shipment. As these factors may influence infection by *A. solani,* the limits of temperature and humidity within which infection may occur and the relation of mechanical injury to infection are of considerable importance in the study of the disease on field grown seedlings.

TEMPERATURE AND HUMIDITY

The influence of temperature and humidity on infection by *Alternaria solani* has been shown by Heald (1) and Rands (3, 4) for potatoes, and by Rands (4) and Weber (5), for tomatoes, in reports dealing with the maturing plant. Kreutzer and Durrell (2) showed that this organism can produce collar rot on tomato seedlings at temperatures greater than the optimum for good plant growth but they did not study the leaf-spot phase of the disease. Both Rands (4) and Weber (5) report that periods of high humidity are followed by the appearance of abundant infection and that fairly high temperatures also are most conducive to the development of the disease.

In the course of investigations of the leaf spot and stem canker caused by *Alternaria solani* on tomato seedlings in southern plant fields, data have been recorded on the effect of different periods of high humidity on the development of leaf spot in order to ascertain the duration necessary for an appreciable amount of infection. Studies also have been made with regard to the lower range of temperatures at which leaf-spot infection may occur.

In order to determine the influence of varying periods of high humidity on leaf-spot infection, several tests were conducted in the greenhouse in which 4 sets of inoculated tomato plants were held at a fairly uniform temperature. Three of these sets were incubated for 4, 8, or 16 hours' time in moist chambers having an atmosphere of 98–100 per cent relative humidity. During the time that the plants were not in the high-humidity chambers, they were held in a greenhouse atmosphere that varied in relative humidity from 40 to 80 per cent. In the temperature studies, the humidity was held approximately constant and the temperature was maintained at different mean levels. Plants of equal age and vigor were used in all experiments. Inoculations were made at the beginning of each test by spraying a heavy spore suspension of *Alternaria solani* over the foliage with an atomizer. Leaf-spot counts were made after an incubation period of 4 to 10 days.

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[1] Cooperative investigations between the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Georgia Coastal Plain Experiment Station, Georgia Agricultural Experiment Station, Georgia Department of Entomology, New Jersey Agricultural Experiment Station, and Indiana Agricultural Experiment Station.

The influence of prolonged high humidity development of leaf spot is shown in table 1. From these data it was found that periods of high relative humidity for as little as 4 hours were sufficient to induce appreciable leaf-spot incidence, but no infection occurred when inoculated plants were held at the lower humidities of the open greenhouse. Disease development increased, though not proportionately, as the hours of high relative humidity were increased. Daily observations of all lots of plants in these experiments showed that humidity sufficient to cause an accumulation of dew on the tomato foliage is necessary for infection by *Alternaria solani.*

TABLE 1.—*The development of A. solani leaf spot on inoculated tomato plants following incubation in an atmosphere of 98–100 per cent relative humidity for numbers of hours shown.* (*Eight 3-plant cultures per treatment in each experiment*)

Test numbers	Date inoculated	Date counted	Mean number of leaf spots per culture			
			0 Hours	4 Hours	8 Hours	16 Hours
1	2/14/41	2/20/41	0	5.3 ± 0.8[a]	47.8 ± 18.8	285.2 ± 55.5
2	3/10/41	3/24/41	0	3.6 ± 1.0	24.6 ± 5.0	166.7 ± 18.9
3	3/24/41	4/ 4/41	0	6.8 ± 1.6	21.3 ± 2.9	154.3 ± 20.2

[a] Standard error of the mean.

TABLE 2.—*Development of A. solani leaf spot on inoculated tomato plants incubated at a constant humidity level but at different temperatures.* (*Eight 3-plant cultures per treatment in each experiment*)

Test numbers	Date inoculated	Date counted	Mean number leaf spots per culture	
			Mean temperature range 54–62° F.	Mean temperature range 74–82° F.
1	1/23/41	1/31/41	142.6 ± 13.0[a]	446.8 ± 95.6
2	2/18/41	2/26/41	225.3 ± 28.3	278.7 ± 37.8
3	3/18/41	3/26/41	62.6 ± 15.3	190.0 ± 27.0
4	3/28/41	4/ 7/41	95.0 ± 30.7	153.2 ± 24.1

[a] Standard error of the mean.

In tests where approximately uniform humidity was maintained at a level sufficiently high to induce infection by *Alternaria solani,* and the temperature was held at mean levels between 54° and 62° and 74° and 82° F., leaf-spot infection increased as the mean temperature was increased, with a rather abrupt increase at 60° F. or above. From the data shown in table 2, it is apparent that leaf-spot infection in the field, under favorable conditions of humidity, will develop more abundantly as the mean daily temperatures rise during the advancing spring season. It is of particular interest, however, to note that appreciable infection by *A. solani* occurred at the lowest temperatures included in these tests, some of which were materially lower than the daily mean ordinarily experienced in the plant fields of the South. From the standpoint of the commercial grower, this is of considerable importance, since it suggests that infection may take place under all field growing temperatures, provided sufficient moisture is present on the leaves.

FOLIAGE INJURY

In the normal process of harvesting, packing, and shipping tomato seedlings, some injury to the foliage and stems is unavoidable. In view of the fact that both humidity and temperature are quite favorable for infection in the shipping crates, several tests were made to determine what influence foliage injury might have upon increased disease incidence. In these tests, one series of plants suffered no injury, the foliage of another was subjected to a unit (300 g.) quantity of sand per series from a small sand-blowing machine, and that of still another to two units of sand. In no case was there visual evidence of appreciable injury to the epidermis of the leaves after the sand blowing was completed. Following this treatment, all lots were inoculated with approximately equal amounts of a heavy spore suspension of *Alternaria solani* and held for periods of from 5 to 8 days in a moist chamber, where both humidity and temperature favored infection. Leaf-spot infection (Table 3), under the conditions of these tests, was increased by mechanical injury immediately before inoculation. In view of the plant injury caused during certain periods of blowing sands, also the unavoidable injury incident to the normal process of harvesting and

TABLE 3.—*The development of A. solani leaf spot on tomato plants inoculated following different degrees of mechanical injury. (Eight 3-plant cultures per treatment in each experiment)*

Test numbers	Date inoculated	Date counted	Leaf spots per culture after treatment		
			Control	Sand abrasion	
				300 g. sand	600 g. sand
1	1/21/41	1/28/41	107.5 ± 31.5[a]	619.3 ± 76.0	879.7 ± 97.0
2	1/21/41	1/29/41	339.2 ± 39.3	594.8 ± 72.1	743.8 ± 101.5
3	3/3/41	3/10/41	80.8 ± 17.7	96.3 ± 20.4	129.6 ± 17.2

[a] Standard error of the mean.

packing tomato seedlings, it appears that leaf spot may be enhanced at certains times by these factors.

STEM INJURY

From the standpoint of the northern tomato grower, the stem canker phase of *Alternaria solani* infection on tomato seedlings is a matter of much greater importance, than is leaf spot. Since seedling stems, as well as foliage, are invariably subjected to slight mechanical injuries during harvesting and shipping, a series of inoculation tests was made to determine the effect of this injury on subsequent development of stem canker. As in the case of the foliage experiments, certain series were inoculated without injury to the plant stems, others were inoculated after the stems were subjected to a unit (432 g.) quantity of sand per series from a sand-blowing machine, and still others after being subjected to 2 units of sand. All lots were held under the same conditions of temperature and humidity for periods of 3 to 5 days, and then read for stem canker (table 4). From these data, it appears that stem canker development, under conditions of optimum temperature and humidity, is likely to increase because of injury sustained by the plants during harvesting and shipping.

TABLE 4.—*The development of A. solani cankers on tomato plants subjected to different degrees of injury prior to inoculation. (Eight 3-plant cultures per treatment in each experiment)*

Test numbers	Date inoculated	Date counted	Stem cankers per culture after treatment shown		
			Control	Sand abrasion	
				432 g. sand	864 g. sand
1	11/18/40	11/22/40	0	4.8 ± 1.0	16.8 ± 3.2
2	11/25/40	11/29/40	1.6 ± 0.5[a]	83.3 ± 8.7	111.2 ± 13.2
3	1/11/41	1/14/41	0	14.0 ± 7.5	40.8 ± 8.4
4	1/13/41	1/17/41	0	52.0 ± 5.5	88.2 ± 8.2

[a] Standard error of the mean.

DISCUSSION

Most of the tomato seedlings produced in the South for northern shipment are grown during March, April, and May. For most plant areas in the southeast, this period is fairly free from excessive rain and high humidity. In the light of data collected on disease incidence under different humidity conditions, it appears that *Alternaria solani* leaf spot may be expected to become seriously important only during periods of prolonged high humidity. Field observations throughout all sections of the plant-producing area of southern Georgia during the period 1937–1940, inclusive, have indicated the validity of this conclusion (Table 5). Since the mean daily temperatures during the spring months rise at a fairly uniform rate each year, disease incidence may be expected to increase with the advance

TABLE 5.—*The number of days and the mean number of hours per day with 4 or more hours of 90–100 per cent relative humidity at Tifton, Ga., during the March 20 to May 20 period for the years 1937 to 1940 together with observations of general field infection of Alternaria solani*

Year	March		April		May		General field infection by *A. solani*
	Number days	Hourly mean per day	Number days	Hourly mean per day	Number days	Hourly mean per day	
1937	7	6.5	18	8.0	10	5.0	Light
1938	8	9.5	17	7.8	6	7.6	Light
1939	11	11.6	25	10.8	19	10.2	Severe
1940	3[a]	9.3	14	6.7	5	5.2	Light

[a] Record for 5 days.

in temperatures during those seasons when humidity conditions are favorable for infection.

During the process of pulling and packing tomato seedlings, some mechanical injury is unavoidable. Since the plants are packed in wet peat moss, a high humidity is created in the shipping crates, particularly near the center, and this is conducive to disease development. In many instances, the crates are subjected to temperatures that are likewise conducive to infections of both the leaves and stems. In the light of the data on the influence of mechanical injury, it appears that, under the above-mentioned shipping conditions, leaf spot and stem canker on tomato seedlings may be increased through mechanical damage to either foliage or stems.

SUMMARY

The principal limiting factor in leaf-spot infection of tomato seedlings by *Alternaria solani*, is humidity. The critical phase of this humidity appears to be the number of hours per day in which the atmosphere is near saturation rather than the daily mean relative humidity for any given period.

Given humidity conditions suitable for *Alternaria solani* infection, leaf-spot incidence increases with the advance in mean temperature within the range studied (to 82° F.). Appreciable infection develops, however, at mean temperatures below those considered suitable for good tomato seedling growth.

Leaf spot and stem canker increases with the degree of mechanical injury to which the plants are subjected prior to inoculation.

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PHYTOPHTHORA CROWN ROT OF LOQUAT

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The loquat, *Eriobotrya japonica* Lindl., has long been grown in California both as an ornamental and as a fruit tree. According to a 1936 survey (2) there were 147 acres of commercial plantings in the State at that time. It is present also in many park, garden, and street tree plantings. In the city of San Jose, California, a survey showed 231 loquat trees had been planted in roadside parking areas. Despite this widespread planting, the crown rot disease due to natural infection by *Phytophthora cactorum* (L. and C.) Schröt. has not previously been reported or described in California. No record of its occurrence elsewhere in the United States has been found.

HISTORY OF CROWN ROT

Dufrenoy (4), in 1927, isolated a *Phytophthora* from loquat crown-rot lesions in Corsica, which he named *P. omnivora parasitica* forma *eriobotryae*. The following year Nicolas and Aggery (6) reported this fungus as a new parasite of the loquat. Tucker (9) suggested that the name of the organism be changed to *P. parasitica*.

Tucker (10) recorded an isolation of *Phytophthora cactorum* from the loquat in Japan in 1932 by T. Tasugi. In 1937, C. O. Smith (8) reported successful artificial inoculations of the loquat and 41 other species of plants with a pure culture of *P. cactorum,* isolated from *Juglans regia*. Baines (1) successfully inoculated flax and peony plants and two varieties of apple with the culture of *P. cactorum* isolated from loquat by Tasugi.

In July, 1939, two 11-year-old loquat trees in the Subtropical Horticulture orchard at the University of California at Los Angeles were found affected with a crown or collar rot. Isolations from the margins of these crown lesions yielded cultures of *P. cactorum.*[1] One of these trees was the Early Red and the other the Advance variety, both budded on seedling loquat stock.

SYMPTOMS OF THE DISEASE

The Early Red tree had two irregular necrotic bark lesions located on opposite sides of the trunk at the crown or collar. A light scraping of the bark revealed the margins of these affected areas. The outer bark tissues of the small but rapidly advancing areas were discolored several inches beyond the zone in which the entire bark was affected to the cambium. These recently invaded tissues were light brown and appeared watersoaked, without any exudation. Brown streaks in the cambium region extended several inches beyond the margin of the affected areas as in the Phytophthora trunk canker or collar rot of apple trees described by Baines (1). The discoloration of the invaded sapwood and the strong odor of fermentation in the diseased bark, which he mentions, were notable characteristics of this loquat disease. The color of the invaded bark varied from Ridgway's (7) fuscous brown to fuscous black at the surface and from Prout's brown to mummy brown beneath, in contrast with the parrot or cress green color of the surface and the white color of the inner tissues of the healthy bark.

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[1] The assistance of Dr. C. M. Tucker in the identification of the culture is gratefully acknowledged.

The presence of the disease was first detected by the appearance of the secondary symptoms on the Advance tree (Fig. 1). The initial crown-rot areas had merged, completely girdling the trunk at the soil level and had extended upward to the main branches. Defoliation had started with the loss of the older leaves and progressed until only a tuft of small, yellow, half-grown leaves remained at the ends of the terminal branches. The yellowing and dropping of the leaves accelerated the decline of the tree. The development of flower clusters was checked and the fruit set greatly reduced. This tree died and was removed in January, 1940, before any of the fruit reached maturity.

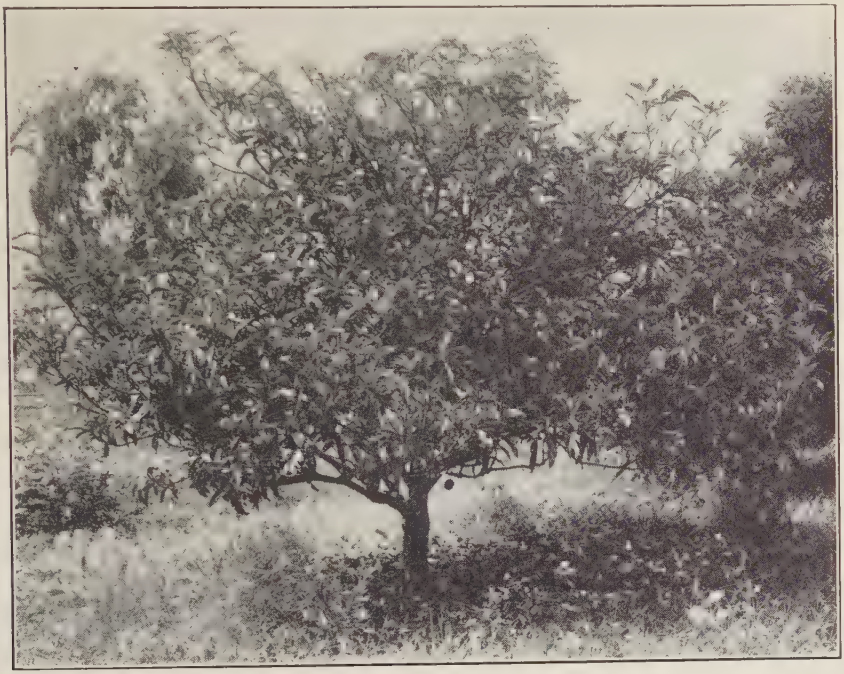

FIG. 1. Advance loquat tree on seedling root girdled by Phytophthora crown and trunk lesions (see Fig. 2). Progressive defoliation has left only clusters of yellowish green, immature leaves at the ends of the branches.

The margins of old inactive cankers on this tree were clearly defined by a bark crack that followed the outline of the lesions. Drying and shrinkage of the dead bark within these areas produced continuous transverse cracks that in some cases extended the full width of the canker. Short, discontinuous, longitudinal cracks broke the surface into small irregular patches of dry, dead bark. On some parts of the trunk these bark scales curled away from the trunk, loosened, and sloughed off, exposing the wood (Fig. 2).

ISOLATION OF THE CAUSAL FUNGUS

Successful isolations of *Phytophthora cactorum* from infected tissues were made by all of the following methods. Direct transfer of small bits of diseased tissue from active canker margins that had been surface-sterilized with 95 per cent alcohol yielded a number of pure cultures. Blocks of tissue from the advancing margins of cankers were dipped in 95 per cent alcohol, passed through a gas flame, and placed in sterile moist chambers. Transfers of the mycelia from these blocks yielded pure cultures. Pure cultures also were obtained from apples inoculated with diseased loquat bark tissue. A fourth method was used to reisolate the fungus from artificially inoculated stems of small seedling loquat trees. Sections of the diseased stems were supported on a wire screen placed on the top of a moisture dish or bell jar filled with water. Dripping water from a faucet splashed over the partly submerged stem sections. Bits of mycelium, which emerged from the diseased tissue, were transferred to agar in culture tubes. Potato-dextrose agar was used for all cultures.

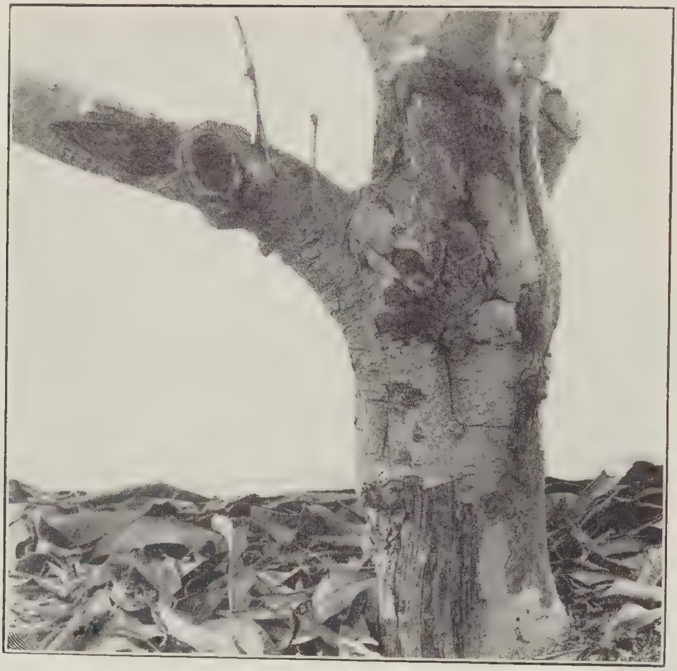

FIG. 2. Advance loquat, 11 years old showing definite margins, bark cracks and bark shedding of old canker that extended around the trunk.

ARTIFICIAL INOCULATIONS

Pure cultures obtained by these various methods were used to inoculate fruits of watermelon, quince, apple, and lemon and seedlings of sweet orange, rough lemon, loquat, pineapple guava (*Feijoa sellowiana*) and natal plum (*Carissa grandiflora*). The fruit inoculations were made by inserting mycelium in rind punctures, each fruit having previously been washed with 95 per cent alcohol and placed in sterile moist chambers. The inoculated fruits were held at room temperature (approximately 70° F.). The method described by Smith (8) was used in the inoculation of the 3 seedlings of each kind. A 3/16-inch cork borer was used to make the inoculation wounds. One check plant was used in each group.

Two weeks after inoculation, the infected areas on watermelon fruits averaged 7 cm. in diameter and showed surface growth of mycelium. Pieces of infected tissue, transferred to sterile tap water in Petri dishes, were held 48 hours at room temperature. Microscopic examination of macerated bits of this tissue showed by that time an abundance of typical antheridia, oogonia, oospores, and sporangia of *Phytophthora cactorum.*

Six days after inoculation, 3 quince fruits had developed infected areas averaging 4 cm. in diameter. Four inoculated apple fruits of the Winesap, Jonathan, Newtown Pippin, Rome Beauty, Bellflower, and Red Delicious varieties, after 9 days, had infected areas averaging from 5 to 7 cm. in diameter. Microscopic examination of rotted apple and quince tissues, after 2 days in sterile water, showed numerous oogonia and oospores but very few sporangia.

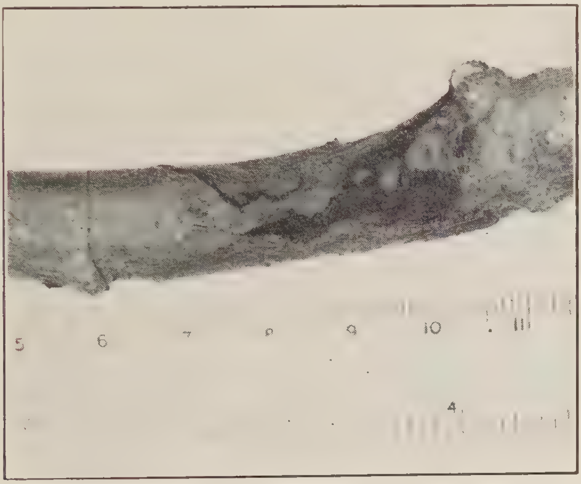

Fig. 3. Typical lesion produced by artificial inoculation of a locust seedling with *Phytophthora cactorum* isolated from naturally infected Early Red loquat tree.

The lemon-fruit inoculations, though repeated several times, were negative, as were those of the seedlings of sweet orange, rough lemon, pineapple guava, and natal plum. Inoculation of 6 loquat seedlings on November 21, 1939, resulted in typical lesions varying in length from 2 to 17 cm. The 17 cm. lesion was measured 45 days after date of inoculation; the others, after 56 days had elapsed. The wounds on the 3 check seedlings had completely healed. One of these typical cankers resulting from an artificial inoculation is shown in figure 3.

DISCUSSION OF ARTIFICIAL INOCULATIONS

Smith (8) reported successful inoculations of lemon fruits and lemon, sweet orange, and loquat trees with the culture of *Phytophthora cactorum* isolated from walnut crown-rot lesions. The negative results of the writer's inoculations with the isolate from loquat suggest that it may be a different physiologic race of *P. cactorum* from that used by other workers. Baines (1) has presented evidence of the existence of physiologic races of this fungus. However, since he also found young Grimes Golden apple trees, 2 to 4 years old, highly resistant to both artificial and natural infection, the negative results from inoculations of the citrus, feijoa and carissa seedlings in this case may not have great significance. It will be necessary to repeat the inoculations using mature trees or plants to obtain further evidence of racial difference.

CONTROL METHODS

Decortication followed by a fungicidal application is the treatment generally recommended (3, 5) and widely used for the control of phytophthora gummosis of citrus trees and crown rot of walnuts. In July, 1939, the crown-rot lesions of the Early Red loquat tree were treated by this method. The bark was scraped lightly to determine the full extent of the infected areas. The dead bark within the areas and the healthy bark 1 to 1½ inches beyond the margins and through the cambium to the wood were removed with a pruning knife. A fungicidal wash of ½ oz. of dry Bordeaux mixture, dissolved in a pint of water, was applied to the treated areas with a brush. In the 2-year period since these areas were treated, neither of them has shown further development.

SUMMARY

A crown rot of the loquat tree, attributable to natural infection by *Phytophthora cactorum*, is described and reported for the first time in this country. Another species of *Phytophthora* had previously been isolated from this host in Corsica and reported as a new parasite of the loquat. *P. cactorum*, isolated from this host in Japan and from walnut crown-rot lesions in California, have been reported able to infect numerous other artificially inoculated host plants.

Irregular brown to black necrotic bark lesions develop at the crown or collar of the tree and extend upward on the trunk and main branches. The bark of old cankers dries, cracks, curls away from the trunk, and sloughs off in patches. In advanced stages of the disease the secondary symptoms appear as a progressive yellowing and dropping of the leaves and general decline of the tree.

Isolation of *Phytophthora cactorum* from the infected tissues was accomplished by 4 different methods. Artificial inoculations of watermelon, apple, and quince fruits and loquat seedlings resulted in infections. Typical cankers developed on the inoculated loquat seedlings.

Decortication, followed by a fungicidal wash, gave effective control of the disease in its early stage.

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THE SUCCESSFUL TRANSMISSION OF PSOROSIS OF CITRUS TREES IN FLORIDA BY BARK GRAFTING

ARTHUR S. RHOADS[1]

(Accepted for publication July 22, 1941)

The convincing demonstration in 1933 and 1934 by Fawcett (2, 3, and subsequent papers) that psorosis of citrus trees is a virus disease engendered an entirely new concept of this peculiar disease that has baffled investigators for many years. The writer has devoted more than a decade to the study of this complex disease in Florida with a view to determining its nature and cause and the possibility of controlling it by the bark-scraping treatment. He has never been able, however, to isolate an organism other than certain ubiquitous fungi commonly associated with it as secondary organisms. In recent years Florida growers have spent considerable time and money in endeavoring to treat psorosis by the bark-scraping method and in making injections of various chemicals into the trunks and limbs of diseased trees, but without success. The writer (6, 7) conducted for 11 years extensive experiments on many trees to determine the effectiveness of bark-scraping in the control of this disease. At the end of this period it could only be concluded that this treatment was very effective in most cases in apparently curing psorosis, or at least temporarily arresting its progress, on orange and grapefruit trees, provided the work be carefully and thoroughly done in the early stage of development of the disease. Even then, however, the disease often developed again in the renewed bark over old treated lesions or at new points on the treated trees in subsequent years. It was found (5) that by the time the disease had reached an intermediate stage of development it almost invariably became systemic and, following the death of areas of the bark, the interior wood of the limbs and trunks of attacked trees usually became extensively invaded and discolored by various secondary fungi that greatly expedited the decline of the trees. These results appear to agree generally with those secured by Fawcett (4) in California and the experience of Doidge and Turner (1) with this disease in South Africa. It is now agreed by all who have given psorosis serious study that it is an infectious disease transmissible through budwood taken from trees that may not show even the bark symptoms of this disease but may be carrying it in a latent form. Usually, from 8–10 years are required for psorosis to manifest itself by the development of the well-known bark symptoms. As Fawcett (2, 3, 4) has shown, however, the leaf symptoms may appear much earlier. Doidge and Turner (1) state that psorosis may continue to develop on individual trees that have been in the orchard for 20 to 30 years, or even longer, without having previously shown bark symptoms. They also state that even psorosis-affected trees that were cut off an inch or two above the bud union and had grown into apparently healthy trees, again developed this disease some 7 years later.

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In 1935, following the presentation by Fawcett (2, 3) of evidence that psorosis is a virus disease, the writer, lacking opportunity to conduct extensive experiments along this line, made a small series of bark grafts in a few large bearing citrus trees. In one of his periodic treatments of trees for the control of psorosis in a grove at Bonaventure, an unusually fine bark lesion 18 inches long was found on an orange limb 4 inches in diameter. The section of the limb bearing this active psorosis lesion was divided into two pieces, one of which was used on May 16 to make a dozen patch-bark inoculations into the upper central limbs of 5 orange trees about 28 years old in a grove near Courtenay on Merritt Island. Pieces of the diseased bark $\frac{3}{4}$-inch square were removed and inserted on the limbs at points where areas of bark of exactly the same size were removed. The pieces of bark thus used as inoculum were held firmly in place with string and painted with melted paraffin. Three inoculations were made on the limbs of each of 3 trees, 2 on limbs of another, and 1 on a limb of a fifth tree.

The other half of this section of limb bearing the psorosis lesion was used for making a series of 20 cultures, of which 17 remained sterile, 1 developed *Colletotrichum gloeosporioides,* 1 *Phomopsis citri* and 1 a slow-growing *Diplodia*-like fungus.

The series of patch-bark inoculations was inspected on October 15, at which time it was apparent that but 6 were successful. These inoculations were inspected at intervals of 2 or 3 times a year, but there was no evidence of the transmission of the bark symptoms of psorosis as late as the end of August, 1937. However, at the next inspection on June 7, 1938, 3 years and 1 month from the date of the inoculations, it was clearly apparent that definite cases of psorosis were developing from some of the successful patch grafts on 3 of the 5 trees. At this time 2 of the 3 inoculations on tree No. 1 showed the typical beginnings of psorosis, the single inoculation on tree No. 2 showed a single small scale of bark at the upper left corner, and of the 3 inoculations on tree No. 4, 2 showed early stage bark scaling typical of psorosis, and the other had developed merely a slight bark scale at the left side.

At the next inspection on May 18, 1939, evidence of the successful transmission of psorosis was much more pronounced. On tree No. 1 the 2 successful inoculations had developed typical psorosis, but the tree was manifesting that chronic wilt and decline designated as blight, and was removed during the summer. The single successful inoculation on tree No. 2 showed a single large scale of bark partly exfoliated at the upper left corner. On tree No. 4 all 3 inoculations showed lesions about 3 inches long and extending $\frac{2}{3}$ around the limbs.

In the inspection of June 10, 1940, the single successful inoculation on tree No. 2 showed more bark scaling, and typical psorosis had developed at the left side of the inoculation. On tree No. 4 a characteristic psorosis lesion extended all the way around one limb, $\frac{2}{3}$ the way around another, and a slight lesion had developed on the other inoculated limb.

At the inspection on August 27, 1940, just prior to the closing of the Citrus Field Laboratory at Cocoa, the single successful inoculation on tree No. 2 showed a typical psorosis lesion 2 in. long and $1\frac{1}{2}$ in. wide at the left side of the inoculation. On tree No. 4 the lesion on the $2\frac{1}{2}$-in. limb on the west side had attained a length of 5 in. and just about completely encircled it, with very pronounced bark scaling and another separate area of bark scaling developed slightly above. On the inoculated $2\frac{3}{4}$-in. limb on the northwest side a typical psorosis lesion 3 in. long and extending part way around it, mostly on the left side of the inoculation, had developed. The third limb, which was 2 in. in diameter, had developed a typical psorosis lesion 6 in. long and completely encircled it. This was removed and photographed (Fig. 1).

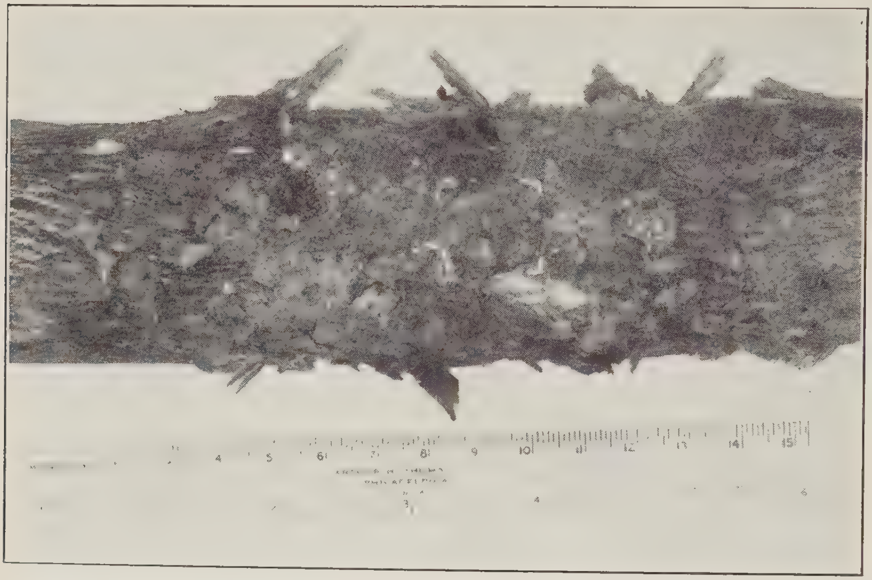

FIG. 1. Typical bark-scaling lesion of psorosis 6 inches long and completely encircling 2-inch orange limb; this developed 5 years after grafting $\frac{3}{4}$-inch square patch of bark (indicated in center) from lesion on another orange tree.

At the final inspection on June 10, 1941, the psorosis lesion on tree No. 2 had attained a maximum length of 3 in. and extended half way around the $2\frac{1}{2}$-in. limb on the left side of the inoculation. On tree No. 4 the lesion on the $2\frac{1}{2}$-in. limb on the west side had completely encircled it and attained a maximum length of 6 in. on one side, with a separate small scaling area above. The lesion on the $2\frac{3}{4}$-in. limb on the northwest side extended half way around it, mostly on the left side of the inoculation, and had a maximum length of 4 in.

It is apparent from the foregoing that psorosis has been definitely transmitted by bark grafting in 6 out of 12 inoculations and the typical bark symptoms of the disease reproduced, their development first being apparent after the lapse of approximately 3 years. Even after the lapse of 3 additional years, however, this slow-developing disease had made very little progress. No bark symptoms of psorosis developed at any points on the 5 trees used in the experiment other than where inoculations were made, and no evidence of psorosis developed in any of the 6 inoculations where the patch of bark used as the inoculum failed to unite with the tissues of the tree. This inoculation experiment furnishes definite proof that psorosis is an infectious disease that may be transmitted from diseased to healthy trees by union of tissues and confirms the work of Fawcett, who has demonstrated that the causal agent is a virus.

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PHLOX RESISTANT TO POWDERY MILDEW[1]

E. B. MAINS

(Accepted for publication July 25, 1941)

The powdery mildew of phlox is placed by Salmon[2] in the species *Erysiphe cichoracearum* DC. It is rather widespread on garden varieties of both the perennial (*Phlox paniculata* L. and *P. maculata* L.) and the annual phlox (*P. drummondii* Hook.). In the vicinity of Ann Arbor, Michigan, it ordinarily becomes noticeable early in the summer and is usually severe by late summer. It may cause considerable defoliation. When this occurs for several years the plants are much weakened. Even when the disease is less severe, the mildew makes plants unsightly, a very undesirable condition for an ornamental.

PERENNIAL PHLOX

In 1933, mildew was unusually severe in the writer's garden. Between several named varieties of perennial phlox there were a number of seedlings. It was noted that one of the latter remained free from mildew throughout the season, while the other plants were severely infected. In the spring of 1934, a division of the resistant plant was transplanted to the greenhouse of the Botanical Garden of the University of Michigan. Here it was studied in comparison with 22 named varieties.[3] In addition 25 seedlings occurring in the vicinity of the resistant plant, and apparently from seed produced by it, also were tested. These plants were repeatedly inoculated with mildew from May through August. Very pronounced differences occurred. All the named varieties were more or less susceptible. The varieties Africa, Commander, Ethel Pritchard, Graf Zeppelin, Lillian, Maréchal French, Miss Lingard, Mrs. W. Van Bueningen, Nicholas Flammel, Paladin (Fig. 1, D). Rijnstroom, R. P. Struthers, Thor, Von Lassburg and Widar were very susceptible. By the first of August, most of their leaves had been killed. The varieties Columbia (Fig. 1, C) Enchantress, Emain Macha, Leo Schlageter, Pastel, Saladin, and Milly Van Hoboken were less susceptible, mildewing somewhat more slowly. By the end of the season their leaves were covered with the fungus. The original resistant selection (H 1) was highly resistant (Fig. 1, A) throughout the entire period. No mildew was evident and the leaves were in good condition at the end of the season. Of the seedlings assumed to have been derived from the resistant plants, 4 were very susceptible, and 4 moderately susceptible. A limited amount of mildew developed on 7 plants. Nine seedlings were highly resistant (Fig. 1, B), showing little or no mildew throughout the study.

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[1] Paper from the Department of Botany, Botanical Garden and Herbarium of the University of Michigan.

[2] Salmon, Ernest S. A monograph of the Erysiphaceae. Mem. Torr. Bot. Club. **9**: 1–292. 1900.

[3] The results were briefly reported at the 27th annual meeting of The American Phytopathological Society at St. Louis, December 31, 1935. Phytopath. **26**: 101. 1936.

In 1935, the varieties Columbia, Emain Macha, Miss Lingard, Nicholas Flammel, and Paladin and the most promising resistant selections from the previous studies were planted outdoors in the Botanical Garden where most of them have been under observation since. In 1935, powdery mildew was severe. The reactions of the varieties and selections agreed very closely with those obtained in the greenhouse in 1934. In 1936, the writer was in British Honduras during the summer, and notes were not obtained. In 1937, mildew did not appear until late, and developed slowly. The most susceptible varieties and selections of previous years became heavily mildewed. Moderately susceptible and moderately resistant varieties showed less mildew and the very resistant selections of previous years continued to be so.

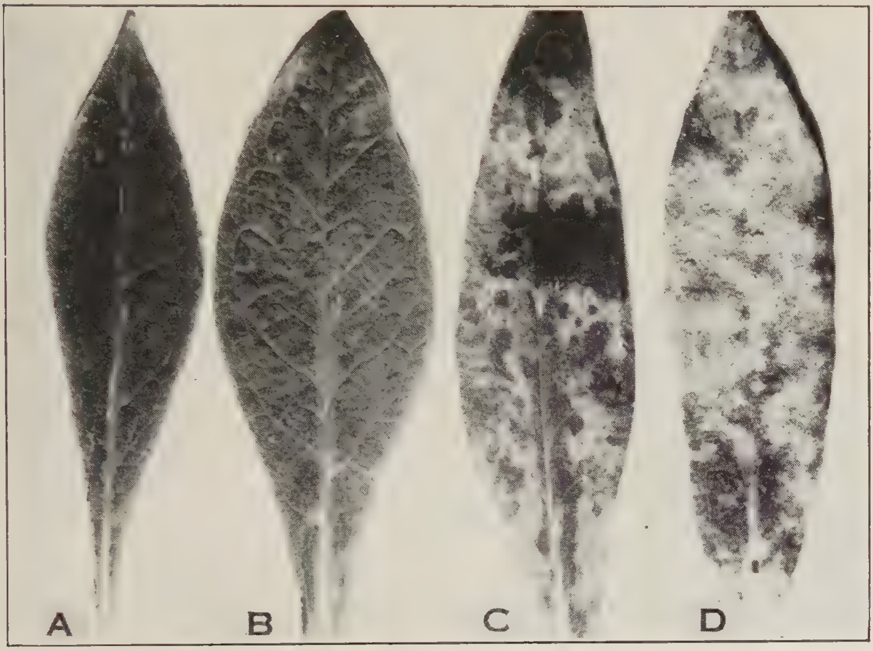

FIG. 1. Leaves illustrating reactions of selections and varieties of perennial phlox to powdery mildew, as the result of inoculations in a greenhouse in 1934. A. Selection H 1, very resistant (compare Fig. 2, B). B. Selection BP 19, very resistant (compare Fig. 2, C). C. Columbia, moderately susceptible. D. Palladin, very susceptible.

In 1938, mildew was again prevalent, commencing early and developing rapidly on susceptible strains. Important changes in reaction occurred. All the original resistant selections developed some mildew. The development was slow and only a moderate infection was reached. They were classified as moderately resistant; however, the differences in reactions, when contrasted with the freedom from mildew of previous seasons, were pronounced. In 1939 and 1940, red spider was so severe in the planting that mildew reaction could not be determined accurately.

In 1941, mildew again appeared early and developed rapidly; red spider was not troublesome. The varieties and selections were subjected to a severe test with very interesting results. Several of the original highly resistant selections (DP 17, 19, 20) were heavily mildewed (Fig. 2, C). Others (H 1, DP 22, 24) were moderately resistant, as in 1938 (Fig. 2, B). Several (DP 4, 24) showed a high resistance (Fig. 2, A). The results for a selected number of the selections and varieties are summarized in table 1. The results of the 1938 and 1941 tests strongly indicate that several physiologic races of the mildew fungus occur.

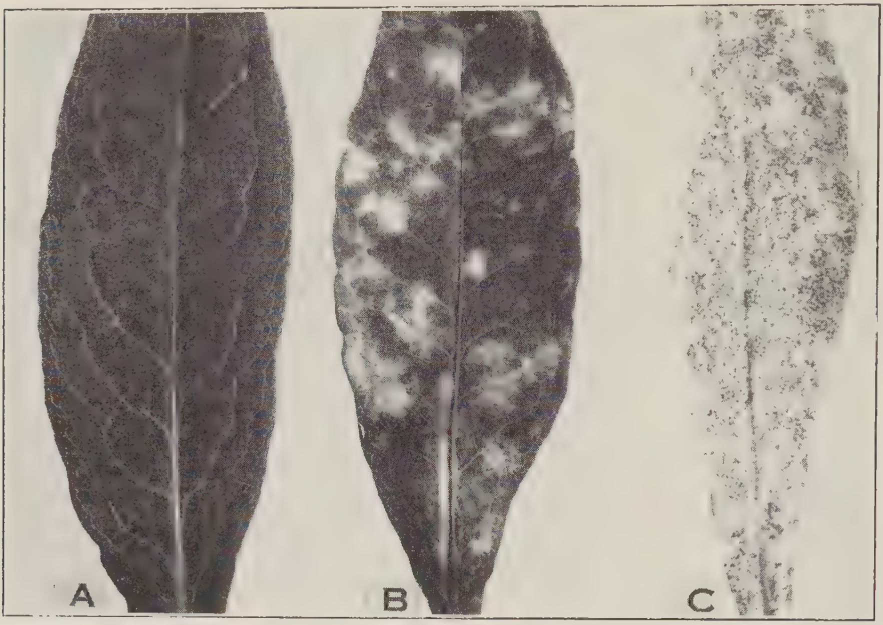

FIG. 2. Leaves illustrating reactions of selections of perennial phlox to powdery mildew in field plantings in 1941. A. Selection DP 4, very resistant. B. Selection H 1, moderately resistant (compare Fig. 1, A). C. Selection BP 19, very susceptible (compare Fig. 1, B).

Although no selection has remained entirely mildew-free throughout this study, several have been more or less resistant. Selection is being continued, with promising results. It should be noted that resistant plants in these studies were exposed constantly to inoculation from adjacent heavily mildewed plants. Much more mildew developed on the resistant plants than would have occurred had they been isolated. In the latter case, whenever the resistant plant became infected through wind-borne spores, the resistance of the selection would have reduced the production of inoculum and thereby greatly retarded the development of the disease. For garden purposes, therefore, the moderately resistant varieties should prove very satisfactory provided susceptible varieties also are not grown.

A number of the resistant selections have mallow purple (Ridgway) flowers, including the original selection which is a very vigorous grower, attaining a height of 64 in. under favorable conditions. Several have well-arranged, pure white flowers of good size. Others have white flowers with deep pink "eyes." Some of the recent selections are deep pinks of good quality. There is also considerable range in height of plants and dates of blossoming. The occurrence of physiologic races of the pathogen complicates the problem of obtaining mildew-resistant varieties of phlox. The results, however, thus far secured indicate that varieties of various types can be obtained with considerable general resistance.

TABLE 1.—*Reactions of a selected number of selections and varieties of perennial phlox to powdery mildew*

Selection or variety	Reaction in[a]				
	1934	1935	1937	1938	1941
H 1	0	0	0	2	2
DP 4	0	0	0	2 +	0
DP 17	1	1	0	2	4
DP 19	0	0	0	2	4
DP 20	0	0	0	3	4
DP 22	0	0	1	2	2
DP 24	0	0	0	2	1
Miss Lingard	4	4	4	3	......
Columbia	3	3	3	4	3

[a] 0 = very resistant, no mildew or only a trace.
1 = resistant, infection scattered and poorly developed.
2 = moderately resistant, development slow and the maximum a moderate amount.
3 = moderately susceptible, development slow, the plants finally well covered.
4 = very susceptible, development rapid, the plants early well covered.
1934 results from inoculation in greenhouse.
1935, 1937, 1938, 1941 results from field plantings.

ANNUAL PHLOX

In 1935 the study was extended to include annual phlox. In order that the chances for the discovery of resistant individuals might be as great as possible, mixed seed of various types was purchased. From this, 606 plants were grown in a greenhouse and inoculated with powdery mildew. Of these, 480 were classed as susceptible, 68 as moderately susceptible, and 58 as moderately resistant. Seed was obtained from 35 of the moderately resistant plants and plants grown and inoculated in 1936. Of these, 9 lines were outstanding. From these, 156 selections were made and seed obtained. The progenies of these were inoculated in the greenhouse in 1937 and 8 lines were found uniformly highly resistant. Of these, Strain No. 67–25 is the most promising. In 1941, 91 plants of this line were inoculated in the greenhouse with mildew; all were highly resistant (0-trace). In comparison 168 plants of a commercial strain were susceptible (3–4). Strain 67–25 has large flowers, with a color between pomegranate purple and rose-red (Ridgway) with a large white eye.

SUMMARY

Selections of perennial phlox have been obtained that are highly resistant to powdery mildew. These include a number of color types.

Through repeated selection in annual phlox several lines have been obtained that are highly resistant to powdery mildew.

Field evidence indicates that several physiologic races occur in the mildew differentiated by the reactions of selections of perennial phlox.

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USE OF ERADICANT SPRAYS FOR THE CONTROL OF ASPARAGUS RUST

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J. P. FULTON

(Accepted for publication July 31, 1941)

INTRODUCTION

Development of rust-resistant varieties of asparagus has been considered a solution of the rust problem in America; consequently, little attention has been given to the control of this disease by other means since the introduction of the Mary and Martha Washington varieties. Resistance in these varieties, however, was known to be near the margin of safety, since rust would often appear in a mild form under conditions favorable for its development. Furthermore, according to recent correspondence with Lawrence Ogilvie[1] of the Long Ashton Research Station, University of Bristol, British and German pathologists consider these varieties only moderately resistant.

During the last 4 or 5 years asparagus growers in Illinois have become alarmed at the amount of rust developing in their fields. This condition has been noted especially in areas of large production. Most of these growers have made an effort to establish their beds from seed or plants certified to be of the Washington varieties. Attempts to control the rust by the use of sprays and dusts have proved ineffective or injurious.

Knowledge of the life cycle of *Puccinia asparagi* DC. led the writers to believe that it might be possible to control the disease by the use of eradicant sprays. There is no good evidence that the rust is perennial, and the only method of initial infection in the spring is from the sporidia of the overwintering teliospores. An efficient eradicant spray, applied during the dormant season, should prevent germination of the teliospores and thus eliminate spring infection. Control under these conditions probably would be local, since a focus of aecial development in the neighborhood of the protected field would soon bring about a general infection from the repeating uredinial stage of the rust. However, it is the opinion of experienced growers that late summer infection does not reduce growth to any extent and, consequently, is of little importance, while early infection results in severe stunting.

The problem of control is simplified in commercial plantings by the fact that in established beds cutting continues until after the period of primary infection by basidiospores. Thus aecia never appear on the plants in harvested fields in northern Illinois, where all the stalks are cut up to the last of June. Young beds left either uncut or cut for only 2 weeks are exposed to the initial infection and thus furnish inoculum for neighboring beds after cutting is discontinued. The main problem, therefore, is to control the rust on these unharvested fields in order to prevent or delay secondary infection.

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[1] Letter to H. W. Anderson, dated March 9, 1940.

The general problem of asparagus-rust epidemiology and control is being reinvestigated by one of the writers (J. P. Fulton). One phase of this problem, the use of eradicant sprays, is here reported, since significant results were obtained that may be of use to other investigators.

PLAN OF THE EXPERIMENT

At Urbana, the plots were located in a field of asparagus that had had a record of heavy rust infection for the past 3 years. Conditions were ideal for secondary infection, since the experimental work on production was based on planting 3 to 4 rows each year and the new plantings were left uncut for 2 years. Thus the harvested rows were always exposed to secondary infection from the new plantings, which always developed abundant aecial pustules. The area selected consisted of 22 rows approximately 250 feet long ranging in age from 3 to 9 years. Rust was evenly distributed over these plots during the summer of 1940, although no exact record of infection was made. Telial material could be collected at any point in the field during the winter months.

TABLE 1.—*Summary of treatment and results of Elgetol eradicant spray experiments at Urbana, Illinois*

Plot[a]	Cultural[b] treatment	Concentration of Elgetol	Dosage per acre	Time of application	Stalks in[c] 50 hills showing aecia June 30	Type of[d] infection
No.		*Per cent*	*Gal.*		*No.*	
1	Uncut	1	800	12/ 5/40	1	L
2	"	1	800	3/27/41	4	L
3	"	None	None	....	68	H
4	"	2	800	12/ 5/40	47	L–16 H–31
5	Cut	2	800	12/ 5/40	22	L
6	"	1	800	12/ 5/40	12	L
7	"	2	800	3/27/41	8	L
8	"	1	800	3/27/41	7	L
9	"	1	400	3/27/41	8	L
10	"	½	400	3/27/41	2	L

[a] Plots, except 2 and 3, were 25 feet wide, running across 22 rows; Plots 2 and 3 were 50 feet wide, across 11 rows.

[b] "Cut" means old plants were cut off and burned prior to treatment; "Uncut" means that old plants were left until after the spring application.

[c] Fifty hills represents about 300 stalks.

[d] L = light, 1–5 pustules per stalk; H = heavy, 6 or more pustules per stalk.

The material used as an eradicant spray was Elgetol (sodium dinitro-o-cresylate). The plot arrangement, concentration of Elgetol, and date of application are given in table 1. A power sprayer with a Bean Spraymaster gun was used, and the pressure was maintained at 400 lb.

A quiet day was selected for both applications, so that there was little drift. As indicated in the table, in some of the plots the old plants were removed before application. This was done a few days before the December spraying, when the stalks were thoroughly dry. The plants were raked together and burned, but many stubs from 4 to 12 in. high were left, and abundant telial pustules were observed on these and on débris (''needles'' and small branches scattered over the surface of the ground). The object of cutting off the old plants was to facilitate spraying, rather than to eliminate the source of inoculum.

Dosage was based on previous experience with ground sprays in apple orchards. A very thorough wetting of the surface can be obtained at 400 gal. per acre, when the old plants are removed; but, when present, 600 to 800 gal. per acre are required to thoroughly soak all the standing plants and to cover the ground.

The concentrations employed were selected on the basis of previous laboratory and field work on apple scab. The dates of application were selected on the basis of cultural practices, which need not be explained at this time.

In the spring a portion of each plot was left uncut. The remainder was harvested until after initial infection has ceased.

A somewhat similar experiment on a much larger scale was conducted in northern Illinois. The results of these experiments will be published later, but the data secured show a remarkably close correlation with those at Urbana.

RESULTS

Microscopic examination of the teliospores a few weeks after the spring treatment revealed typical collapsed protoplasm, such as had previouly been observed in other fungi. A few spores in each sorus appeared normal, and some sori had as many as 10 per cent of what appeared to be normal spores. Data on germination of the normal and treated teliospores were collected, but will not be presented at this time.

The first aecial pustules were observed during the first week in May. April was dry, excepting the 15th to 20th, when a very heavy rain occurred. Shoots were tagged as they appeared above ground, and, judging from the infection records on these, there was only one extensive infection period, which must have occurred shortly after this rainy period. A 2-inch rain on May 6 apparently did not result in renewed infection, although weather conditions seemed ideal. A like limited infection period prevailed at Rochelle, where the larger experiments were conducted. Because of the limited number of aecial pustules this season, the results obtained from the eradicant sprays are not so conclusive as could be desired.

Records on the number of stalks showing aecial pustules were taken at various times during the spring. A final count on June 30 is recorded in table 1, and will serve as a basis for comparison. It is evident that all of the treatments gave considerable reduction in infection, and that, with the exception of plot 4, there would appear to be no great difference in the effectiveness of the treatments regardless of concentration, dosage, time of application and previous treatment of the old plants. The contrast between the check and treated plots was especially noticeable when one examined the number of infections (pustules) per stalk. In the treated plots infections usually consisted of 1 or 2 pustules per stalk, while in the check plot nearly every stalk showed numerous pustules, indicating multiple infection.

Plot 4, in spite of a very excellent treatment, shows much higher infection than the other treated plots. Evidently this was due to its being located next to and north of the check plot. This conclusion was further supported by the fact that most of the infection on this plot was at the south end immediately adjoining the check plot.

Observations were made on the spread of the rust as evidenced by the development of the uredia. The data on this phase of the work will be presented in a future publication, but, in general, it was evident that by June 30 the uredia were developing very extensively over the check plot, but were limited to a few areas in the treated plots near the stalks showing aecia, or adjoining the check plot. By July 15 one could see the outline of the check plot at a distance, due to the prevalence of brown plants, while in the treated plots only an occasional infected area could be observed. Adjoining the plots were several rows which had been harvested in April and May, but had been allowed to grow after the cutting season. Plants in these rows were becoming heavily rusted where they adjoined the check plot, but no rust was found on those adjoining the treated plot on July 15.

DISCUSSION AND CONCLUSIONS

An eradicant spray, to be effective against asparagus rust, must reduce teliosporic inoculum to such an extent that few aecial pustules develop, since secondary infections from aeciospores and urediospores build up rapidly. The limited results obtained this season indicate that such a reduction may be secured by the application of Elgetol at concentrations of from $\frac{1}{2}$ to 2 per cent and dosages from 400 to 800 gal. per acre during the dormant season. There seems little difference as to efficiency of fall or spring applications, and the time selected should be governed by the cultural practices of the grower.

It is recognized that a problem exists in areas where large plantings of asparagus are near escaped plants or small garden plantings that are not cut during the early part of the season. Even under such conditions, secondary infection is delayed and is frequently local.

Progressive commercial asparagus growers have abandoned the practice of burning off their beds, since they wish to conserve the humus. Futhermore, burning is not effective in destroying all the inoculum, since observations prove that stubs often are left and the infected needles, which drop to the ground during the growing season, often are protected during the burning of the bower. The application of an eradicant spray allows the grower to retain the humus-building plants without danger of increasing infection.

Treatment may be confined to young beds, which are not to be harvested the following spring. Under such conditions the cost of the eradicant spray program is not excessive, since, usually, only a small proportion of the total acreage is in young beds.

It is recognized that the ultimate solution of the rust problem in asparagus is the development of more highly resistant strains of our present rust-resistant varieties; but, while awaiting this development, an effective and economical control is believed to have been discovered in the use of an eradicant spray.

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AN ELSINOË CAUSING AN ANTHRACNOSE OF VIRGINIA CREEPER

Anna E. Jenkins and A. A. Bitancourt[1]

(Accepted for publication August 7, 1941)

The presence of an *Elsinoë* on Virginia Creeper (*Parthenocissus quinquefolia* (L.) Planch.) has recently been shown through the discovery of a fungus of this genus on severely diseased leaves, stems, and fruits of this vine growing as an ornamental in the vicinity of Marlboro, New Hampshire.

On August 31, 1940, representative specimens from this source were sent for diagnosis to the Forestry and Recreation Department of New Hampshire, whence they were referred to the Laboratory of the Division of Forest Insect Investigations, U. S. Department of Agriculture, at New Haven, Connecticut, and next, to Alma M. Waterman at the New Haven headquarters of the Division of Forest Pathology of the same department.

Numerous small lesions were present on leaves, stems, and fruit. On discovering that an ascomycete, apparently an *Elsinoë*,[2] was fruiting abundantly on the leaf lesions, Dr. Waterman forwarded the material to the senior writer for further study. The identification was confirmed, as recently noted by Dr. Waterman.[3]

Following the receipt of the mature material from New Hampshire (Fig. 1, A and B), an examination was made of phanerogamic specimens of Virginia Creeper in the U. S. National Herbarium. Abundant infection by the *Elsinoë* was present on a specimen from Florida bearing the date July, 1891. This was from the Chapman Herbarium, having been collected by Dr. Chapman at Apalachicola, where he lived[4] during the latter part of his life.

The *Elsinoë* on Virginia creeper, a new host, is here described as new under the proposed name of *E. parthenocissi.*

Leaf spots few to numerous, generally originating on the upper leaf surface where they are the more distinct, consisting of well delimited circular to subcircular or occasionally irregular areas 0.2–4 mm. in diam., scattered or often distributed along the midrib and veins, or sometimes involving them, where numerous, often forming a more or less coalescent row of lesions serving to outline part or all of the more prominent venation, on dry specimens individual lesions generally depressed below and raised above, with

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[1] Paper presented by the senior writer on June 25, 1941, at Windsor, Connecticut, during a session of the summer meeting of The American Phytopathological Society held jointly with the New England branch.

[2] Jenkins, A. E., and A. A. Bitancourt. Revised descriptions of the genera *Elsinoë* and *Sphaceloma.* Mycologia 33: 338–340. 1941.

[3] Waterman, A. M. Diseases of shade and ornamental trees: annotated list of specimens received in 1940 at the New Haven Office, Division of Forest Pathology, Bureau of Plant Industry. Plant Dis. Rptr. 25: 181–186. 1941.

[4] Mohr, C. Alvin Wentworth Chapman. Bot. Gaz. 27: 473–478. 1899.

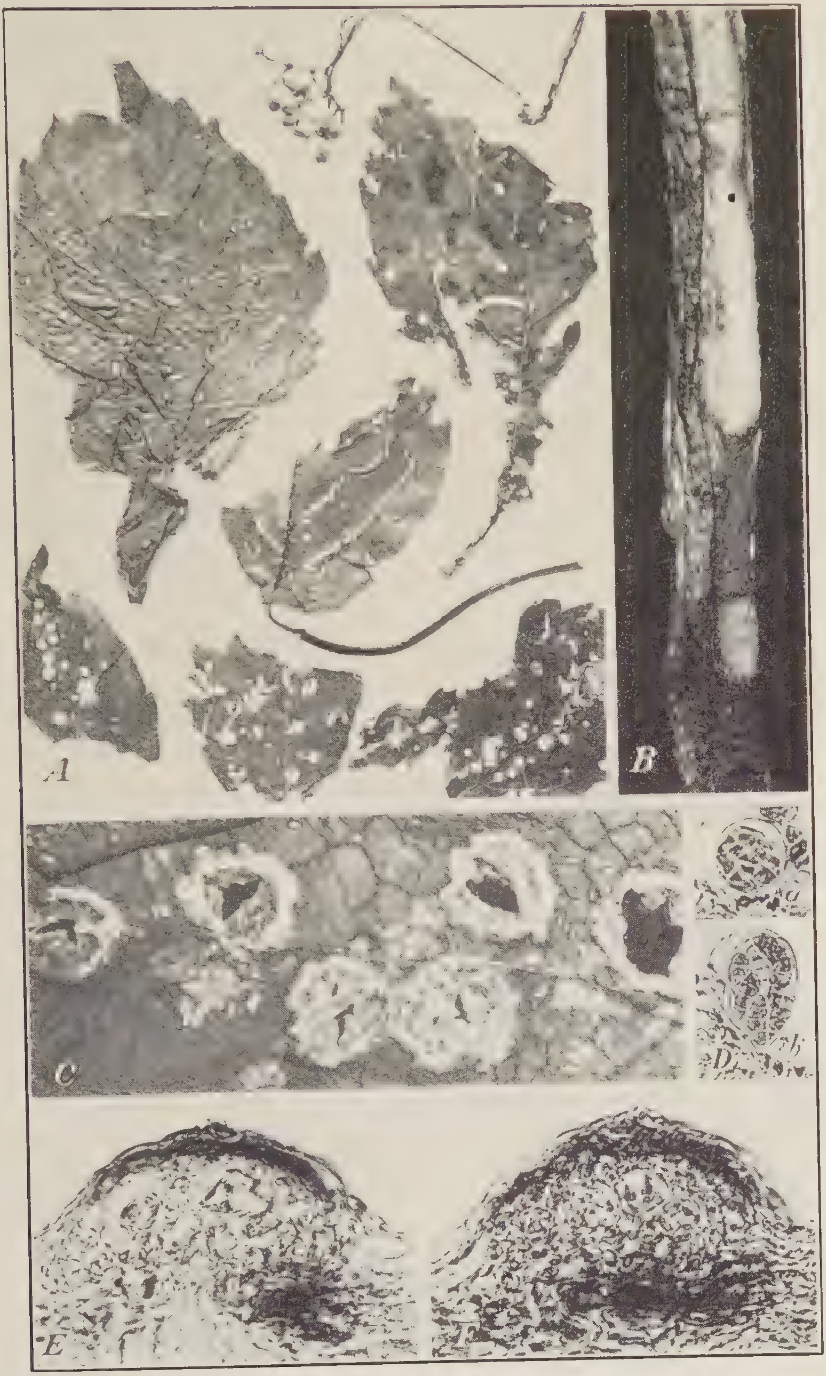

FIG. 1. A. *Elsinoë parthenocissi* on *Parthenocissus quinquefolia*, Marlboro, N. H., Aug. 31, 1940, Mrs. P. S. Howe (type). ×1. B. Stem cankers from lower part of fruiting shoot shown in A. ×10. C. Enlargement of leaf spots, along a vein, upper surface, showing dark fructifications of the *Elsinoë*. ×12½. D. Two asci (a and b) containing ascospores, outer inelastic wall of the ascus in *b*, ruptured and thicker elastic inner wall somewhat expanded. ×500. E and F. Two ascomata showing asci and dark epithecium. ×500. Photographs by M. L. F. Foubert (A–C) and by A. A. Bitancourt (D–F).

generally flattened "vinaceous buff"[5] surface surrounded by a narrow "clove-brown" margin, spots sometimes traversed by prominent fine dark lines representing the smaller venation, part or all of the lesion easily falling away, leaving a shot-hole effect; petiole and stem cankers more or less raised, usually with dark margins and lighter-colored centers; lesions on fruits grayish-white (Waterman); disk also infected; ascomata abundant on the necrotic spots in living leaves amphigenous but more commonly above, at first, intraepidermal; later, penetrating more deeply in the leaf tissues, composed of a hyaline or slightly yellowish pseudoparenchyma of isodiametric, somewhat thick-walled cells; ascomata 70–160 μ in diam., and 35–80 μ in thickness; asci mostly globose, irregularly embedded in the pseudoparenchyma, with thin outer wall, and thick inner wall, particularly at the apex, 17–25 μ in diam., containing 8 ascospores; ascospores 3-septate, constricted at the septae, especially at the median septum, occasionally with a longitudinal septum in one of the middle cells, straight or somewhat curved, with the upper cells broader and shorter than the lower ones, 12–17 by 6–8 μ. Ascospores larger than those of *Elsinoë ampelina* (de Bary) Shear[6] on *Vitis*, which, besides *E. viticola*, is the only other species known on Vitaceae, and is represented only by a single discovery on overwintered cankers on Niagara grape. Additional material of these fungi will be necessary for an adequate comparison of them.

Maculae in foliis e parcis numerosae, supra conspicuores, bene definitae, generaliter circulares vel subcirculares, 0.2–4 mm. in diam., sparsae vel per nervum medium et nervos alteros dispositae, in foliis vinaceo-alutaceae, brunneo-marginatae, in fructibus griseo-albae; cancri in petiolis caulibusque plus minusve elevati margine fusco et centro pallidiore; ascomata in maculis foliorum vivorum abundantia, amphigena, supra numerosioria, prominentia, ascomata 70–160 μ in diam. et 35–80 μ crasso; asci plerumque globosi, in parenchymate hyalina vel pallide flavidula irregulariter immersi, 17–25 μ in diam.; ascosporae 3-septatae, ad septa praecipue medio constrictae, cellula media una interdum longitudinaliter septata, rectae vel subcurvatae, cellulis superioribus latioribus et brevioribus, 12–17 μ longis, 6–8 μ latis.

On leaves, stems and fruit of *Parthenocissus quinquefolia* (L.) Planch. (=*Ampelopsis quinquefolia* (L.) Michx. and *Psedera quinquefolia* (L.) Greene), Marlboro, New Hampshire, and Apalachicola, Florida, causing a destructive anthracnose, here so designated in accordance with this term as originally[7] applied to the equivalent disease of grape.

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[5] Colors in quotations are based on Ridgway, R., Color Standards and Color Nomenclature, 43 pp. and 53 colored plates (Washington). 1912.

[6] Shear, C. L. Life history of *Sphaceloma ampelinum*. Phytopath. **19**: 673–679. 1929.

[7] Fabre, E., and Dunal, F. Observations sur les maladies régnantes de la vigne. Bul. Soc. Cent. d'Agr. Dép. de l'Hérault **40**: 11–75. 1853.

Specimens examined:

Apalachicola, Fla., July 1891, A. W. Chapman. (Mycological Collections, Bureau of Plant Industry 73808. This fragment of the phanerogamic specimen from the Chapman Herbarium now filed in the U. S. National Herbarium under accession number 958621, was obtained through the courtesy of W. R. Maxon.)

Marlboro, N. H., Aug. 31, 1940. Mrs. P. S. Howe (type) (Myc. Coll., B.P.I. 73616. Phytopathological Section Instituto Biologico, São Paulo 4022) June 28, 1941, A. E. Jenkins; July 29, 1941, Mrs. P. S. Howe.

BUREAU OF PLANT INDUSTRY,
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ROUGH–BARK, A VIRUS DISEASE OF FLOWERING CHERRY[1]

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(Accepted for publication September 2, 1941)

An abnormal growth condition of the Kwanzan variety of flowering cherry (*Prunus serrulata* var. *Kwanzan*) was called to our attention in 1939. In a nursery planting the trees were dwarfed in height and seldom produced any lateral branches. The bark, instead of being smooth and a normal gray, was deep brown and roughened by longitudinal splitting. The leaves were grouped close together due to a shortening of the internodes, and most of the leaves were arched downward by the improper development of the midrib (Fig. 1, B). These clustered heads of foliage and the curling of the leaves are similar to the effects of aphid colonies on cherry leaves. There is often a longitudinal splitting and cross cracking of the under surface of the midribs of the leaves. Vein clearing and ragged leaves formed by the cutting out of necrotic areas on the young leaves are prominent symptoms on rough-bark trees, but these symptoms are likewise present on most varieties of *Prunus serrulata* that are not affected with the rough-bark condition. This vein-clearing and ragged-leaf condition may prove to be due to another virus or to physiological conditions.

Similar rough-bark symptoms have been observed on two large Kwanzan trees. The leaf symptoms and splitting of the bark of the younger wood is similar to that noted on the nursery trees, but on the older wood the splitting and roughening of the bark are more pronounced (Fig. 1, A). The seasonal growth of these trees is from 6 to 12 inches long, while that of the normal trees is 2 to 3 feet. The symptoms of these trees immediately suggested that the trouble might be of virous origin.

In the nursery where the disease was first observed, there were 592 trees affected with this condition in a planting of 3,887 trees. The trees were grouped in row areas consisting of 8 or 10 consecutive trees, or approximately the same number of trees as the average number of buds obtained from one bud stick. This segregation of abnormal trees suggested a bud-perpetuated disease, and an examination of the mother block where the buds had been taken showed 2 trees with symptoms similar to those of the young trees. The following experiments were conducted to determine the nature of this trouble.

A block of 75 mazzard seedlings, which are commonly used as root-stock for flowering cherries, was divided into 3 lots of 25 trees each. One lot was budded with buds taken from a normal Kwanzan tree; another lot was budded with buds taken from a rough-bark Kwanzan tree; and the third was budded with buds taken from both the healthy and diseased trees. In the latter, the normal bud was introduced near the soil line and the bud from a tree showing rough-bark symptoms was placed on the same seedling but on the opposite side of the stem and 12 inches above the normal bud. The trees were allowed to develop into 2-year-old nursery trees before the final observations were made. The lot budded with normal buds produced 22 well-developed normal trees. The lot budded with rough-bark buds produced only 6 trees, and these were dwarfed and showed typical rough-bark symptoms. Where healthy and diseased buds were placed on the same seedling, both buds developed on 7 of the trees, only the normal bud devel-

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[1] Published as Technical Paper No. 388 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Botany Department.

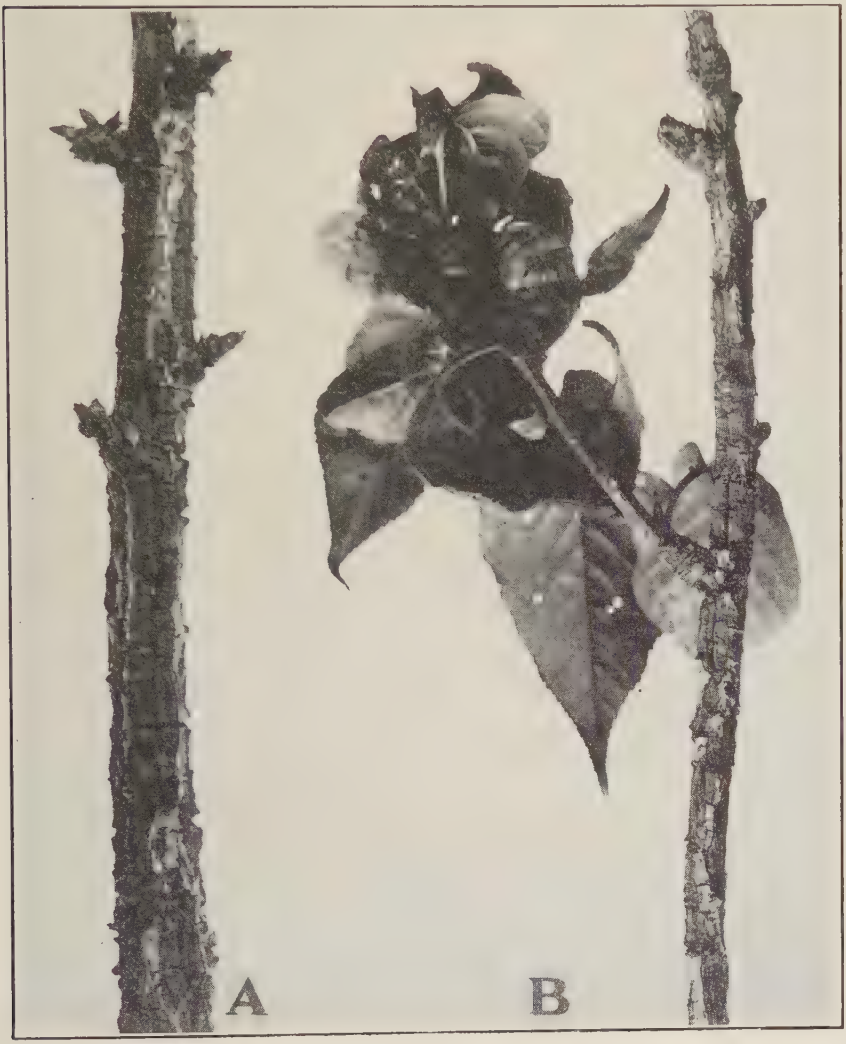

FIG. 1. Rough-bark of Kwanzan flowering cherry. A, Branch from large diseased tree showing characteristic bark symptoms. B, Illustrates rough-bark of stems and downward arching of the leaves on a 2-year-old nursery tree.

oped on 9 other trees, and only the rough-bark bud developed on 3 trees. All growth that resulted from these buds showed characteristic rough-bark symptoms regardless of the type of bud used or its position on the seedling. Of special interest is the group of 9 rough-bark trees produced by the normal bud, even though the rough-bark bud did not develop. This would indicate that the virus passed into the mazzard seedlings from the rough-bark bud, even though organic union was not sufficient for bud development. This was demonstrated again the following year when 24 mazzard seedlings were budded with rough-bark in October. When these buds failed to develop, the same seedlings were grafted with scions from normal trees the following March. Of the 24 trees that developed from the scion wood, 23 showed typical rough-bark symptoms, while adjacent seedlings that had not been previously budded with the rough-bark buds, but were grafted with scion wood from the healthy tree, grew into normal trees. In many instances new shoots of the mazzard seedling have developed from the roots of Kwanzan trees showing the disease, but these sprouts did not show rough-bark symptoms. This indicates the mazzard seedling may become a symptomless carrier of the virus.

These observations and transmission experiments suggest that a virus is concerned. The symptoms produced by the virus on this host are very characteristic and unlike any of the other known viruses of *Prunus*. The common name, rough-bark, is offered for this new disease, and, in the number system, *Prunus virus 9* may be assigned to the virus. For those using Holmes' system of nomenclature, both a new genus and species name is proposed. The genus name of *Rimocortius* is based on the most characteristic symptom of the disease, *bark cracking*. Since the disease seems to be limited to the Kwanzan variety, the name **Rimocortius Kwanzani** is assigned to it.

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MALE-STERILE BARLEY FOR STUDY OF FLORAL INFECTION[1]

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(Accepted for publication August 27, 1941)

A male-sterile barley, suggested as a new tool for the plant breeder,[3] also appears to provide a means for mass inoculation of barley flowers with cultures of disease-producing organisms transmitted by floral infection. The procedure should be particularly useful in studies of conditions affecting infection. At present such studies would necessarily be limited to the few varieties possessing the male-sterile character, but there seems to be no reason to assume this character may not be found in or introduced into other varieties as needed. The male-sterile plants have been shown to be self-sterile, and highly cross-fertile. Ratios of 3 fertile : 1 male-sterile in F_2 populations, and 1 fertile : 1 male-sterile in backcrossed populations demonstrate the simple inheritance of the character. Of particular interest to plant pathologists are the open glumes of the male-sterile plants, which permit rapid inoculation. The degree and persistence of glume opening and of cross fertility have been observed to vary somewhat with the environment. Satisfactory seed sets commonly result from natural crossing and very good seed sets have been obtained with controlled pollination.

Male-sterile barley spikes were inoculated with spores of the organisms causing barley stripe (*Helminthosporium gramineum* Rabh.), which disease is fairly common in California, and of loose smut (*Ustilago nuda* (Jens.) K. & S.), which seldom persists in California, probably because of the dry atmosphere.

In the stripe-infection experiments (Table 1), male-sterile spikes were first pollinated by a mass dusting of the spike with pollen from F_1 hybrid plants that were heterozygous for the male-sterile factor. Then, leaves collected from plants killed by stripe were pulled from the top of the spike downward between each row of glumes to facilitate discharge of spores into the open florets. The inoculated spikes were then bagged. Percentages of infection obtained on the 3 spikes treated were satisfactory, as shown in table 1, and were about equal to any obtained at this station, by the culture of sprouting barley on a medium-base supporting mycelial growth of the stripe organism.

The tests with loose smut involved mass dusting with both pollen and smut spores. Infections of loose smut (Table 1) were rather low. This

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[1] Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Departments of Agronomy and Plant Pathology, University of California, cooperating.

[2] Associate Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture; and Instructor in Plant Pathology and Junior Plant Pathologist in the Experiment Station, respectively.

[3] Suneson, C. A. A male sterile character in barley. Jour. Hered. 31: 213–214. 1940.

TABLE 1.—*Infections obtained with the barley diseases, loose smut (Ustilago nuda) and stripe (Helminthosporium gramineum), by floral inoculation of mass-pollinated male-sterile barley plants at Davis, California*

Pollen parents (F_1 hybrids) used on male-sterile plants	Seeds set on spike	Plants established		
		Healthy	Infected	
	Number	*Number*	*Number*	*Per cent*
Loose smut:				
Male-sterile × C.I. 5636	27	15	3	16.7
Male-sterile × *nudeficiens*	33	26	1	3.7
Do	32	21	1	4.5
Do	33	20	1	4.8
Do	25	17	2	10.5
Male-sterile × Meloy	30	22	0	0.0
Male-sterile × *nigrinudum*	43	28	2	6.7
Do	57	26	12	31.6
Do	20	10	2	16.7
Do	50	34	6	15.0
Stripe:				
Male-sterile × Colsess V	45	2	17	89.5
Male-sterile × *nigrinudum*	52	12	28	70.0
Do	33	5	25	83.3

may have been due to varietal resistance, low atmospheric humidity at the time of infection, or to poor viability of the smut spores. Tapke[4] showed that the viability of loose smut spores is soon lost. The plants from which the smut was collected were headed on April 4, but the smut was not collected and used until April 10 to 17, when most of the spores had been blown away. Among the 30 smutted plants were 5 that produced some disease-free spikes, of which 1 had normal fertility and 4 were male-sterile.

[4] Tapke, V. F. A technique for identifying the loose smuts of barley. Phytopath. 31: 284–286. 1941.

PHYTOPATHOLOGICAL NOTES

A Chemical Method for the Determination of Tobacco-mosaic-virus Protein in Plant Extracts.—Biological assay methods for measuring virus activity are subject to a rather large error. It is impossible to convert virus activity, thus measured, into quantity of virus protein per cc. of extract, even when comparing an unknown against a purified virus preparation of known virus protein content. This is due to variation in the state of aggregation of chemically purified virus preparations and to other factors relating to the plants and process of inoculation.

Martin, Balls, and McKinney[1] described a method for measuring virus protein in tobacco leaf tissue based upon the observation that proteins of healthy tobacco leaves are partly digested by trypsin, whereas the virus protein resists such digestion. About 20 per cent of the nitrogen of leaf tissue of healthy plants was not rendered soluble by tryptic digestion. Because this undigested portion was several times larger than the quantity of virus protein, it appeared likely that this may have introduced an appreciable error in the results.

This problem was studied further, using extracts of healthy and mosaic-diseased leaves in order to eliminate the large fraction of insoluble tissue nitrogen shown incompletely digested by trypsin. Trypsin digestion, when applied to leaf-tissue extracts, left the proteins of healthy tobacco leaf extracts incompletely digested in 24 hours at pH 7.5 and 37° C. The quantity of trypsin-resistant protein varied from 23 to 54 per cent of the total soluble protein, depending upon source of material. Increasing the trypsin concentration from 1 to 5 mg. per cc. of extract did not reduce the amount of undigested protein.

Several methods were studied for separating virus from non-virus proteins of tobacco leaf extracts, including thermal fractionation and use of various protein precipitants and denaturants. The method finally adopted is based upon the observation by Best[2] that tobacco mosaic virus is precipitated from aqueous solutions at its isoelectric point, pH 3.4.

An extract of mosaic-diseased tissue was prepared by adding 1 cc. of M/10 phosphate buffer, pH 7.0, per g. of chopped frozen tissue. After thawing, the tissue extract was pressed out and clarified either by centrifuging or filtering through "Celite." A 40-cc. portion of the clear extract was measured into a 50-cc. centrifuge tube and acidified to pH 4.2 to 4.0 by cautiously adding N/10 H_2SO_4. A large portion of the non-virus protein precipitated during overnight refrigeration was removed by centrifuging at 3000 r.p.m. The supernatant liquid was decanted, further acidified to pH 3.4, allowed to refrigerate overnight, and then centrifuged 30 minutes at 3000 r.p.m. The precipitate was transferred to a 10-cc. centrifuge tube and protein nitrogen was determined by precipitation with 2.5 per cent tri-chloro-acetic acid and digestion and distillation as ammonia in a micro-Kjeldahl apparatus. Since the protein precipitated at pH 3.4 contains virus protein plus a trace of non-virus protein, it is necessary to run a blank on an extract of healthy leaf tissue.

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[1] Martin, L. F., A. K. Balls, and H. H. McKinney. Jour. Biol. Chem. 130: 687. 1939.

[2] Best, R. J. Austr. Jour. Exper. Biol. and Med. 14: 1. 1936.

Accuracy of the method was determined by measuring the quantity of virus-protein nitrogen in extracts of healthy tobacco tissue amended by known amounts of purified virus protein (Table 1).

TABLE 1.—*Recovery of virus protein added to 30 cc. of leaf extract from healthy tobacco*

Virus N added	Virus N recovered[a]	Virus N recovered
mg.	*mg.*	*percentage*
0.305	0.259	85
0.611	0.553	91
1.020	1.030	101

[a] Blank on 30 cc. healthy leaf extract yielded 0.05 mg. N, which figure was used for correcting the results.

The concentration of virus protein in mosaic-diseased tobacco leaves usually is in the range of 0.6 to 3.0 mg. of virus nitrogen per 40 cc. of leaf extract. Within this range of concentration the error of estimation by the above method is less than 10 per cent.

The virus-nitrogen content of extracts of mosaic-diseased tobacco leaves was determined by the foregoing precipitation method and compared with lesion counts obtained from assays on primary leaves of Scotia beans. Comparisons were made against a control inoculated on one leaf of each pair of bean leaves (Table 2).

TABLE 2.—*Comparison of lesion counts and virus nitrogen content of extracts of mosaic tobacco leaves*

Soln.	Virus N per 40 cc. extract	Virus assay[a]		
		Solution (10^{-3})	Control[b]	Per cent of control
	mg.	*lesions/leaf*	*lesions/leaf*	
I	2.23	33.7	74.6	45.17
II	2.04	20.1	59.2	33.95
III	1.53	17.3	54.4	31.80

[a] 30 Scotia bean leaves inoculated with each solution.

[b] Control was identical for each set of 30 plants, and served to facilitate comparisons between the three solutions on a percentage basis.

The isoelectric precipitation method has a smaller experimental error than the biological-assay methods, and can replace them in certain work. The method is rapid and permits expression of absolute quantities rather than as comparative lesion counts, affected by aggregation of infective particles, by host susceptibility and other factors. Biological assays, however, must be used when virus activity is in question, when only small amounts of virus are available, and when the concentration of virus protein is very low.—CLAUDE H. HILLS, Protein and Nutrition Research Division, Bureau of Agricultural Chemistry and Engineering, and H. H. MCKINNEY, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

Transmissible Rough-bark Diseases of Fruit Trees.—Several attempts have been made in recent years to classify viruses, reaching the extreme of applying a Latin binomial ostensibly based on virus characteristics but actually based exclusively on symptoms of the disease. Parallel with this trend and perhaps to some degree because of it, there seems to have been a decline of interest in the orderly grouping of *diseases* caused by viruses.

Most of the virus diseases of plant that have been studied can be and often are placed in one of two large groups, the mosaic and yellows diseases. There appears now to be a considerable number of virus diseases of woody plants, mostly little known, falling naturally into a third group,[1] type have been described, notably psorosis of citrus,[2] stony pit of pear,[3] and diamond canker of prune.[4] The diseases of this group affect the bark primarily, the wood occasionally, the leaves and fruit infrequently. They are extremely slow in development in the tree. The evidence to date indicates a narrow range of susceptible species or varieties for each disease.[4] Thus, trees affected by a transmissible disease of Bosc pear similar to or identical with stony pit have been top-worked to Bartlett, Comice, and Hardy, with no fruit symptoms on the 3 last named varieties up to 5 years for Bartlett and Hardy and 6 years for Comice from the time of grafting. There are indications, also, of more than one disease of this type in a single fruit species.[2] For example, the Bosc disease just referred to produces in Hardy a mosaic pattern in leaves, but no fruit symptoms, while a similar disease occurring naturally in Hardy in the same district is transmissible by grafting from Hardy to Hardy producing fruit pitting but not clear-cut leaf symptoms.

A considerable number of infection experiments have been initiated but at least several more years will be required to bring most of them to a final conclusion. On the basis of symptoms, distribution in the orchard, persistence in cions, and, in a few cases, transmission by grafting, the following diseases found in central and northern California may be mentioned as probably belonging in the rough-bark group. Diseases of the diamond-canker type occur on apricot, cherry (*P. avium*), plum (*P. domestica*), prune, and rose (Pink Radiance) (Fig. 1, C). All of these persist in cions.[5] A disease of Gravenstein apple (Fig. 1, D), known locally as flat limb, is characterized in the apple chiefly by irregularities in wood development, but may be included here because of the rough-bark symptoms produced (Fig. 1, A) when inoculations are made from apple to *Pyracantha.* At least the two diseases referred to above, and apparently more, are found in pears ranging in bark symptoms from measles on younger branches to excessive sloughing of outer bark or "oak bark" on older parts. In addition, the affected Bosc pear may display symptoms (Fig. 1, B) not greatly unlike those of the Gravenstein disease (Fig. 1, D).

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[1] Thomas, H. Earl. Graft transmission persistence and migration of some viruses in fruit trees. (Abstract) Phytopath. **30**: 790. 1940.

which may be designated as rough-bark diseases. Several diseases of this

[2] Fawcett, H. S. Psorosis in relation to other virus-like effects on citrus. (Abstract) Phytopath. **29**: 6. 1939.

[3] Kienholz, J. R. Stony pit, a transmissible disease of pears. Phytopath. **29**: 260–267. 1939.

[4] Smith, Ralph E. Transmission of diamond canker of the French prune. Phytopath. **31**: 886–895. 1941.

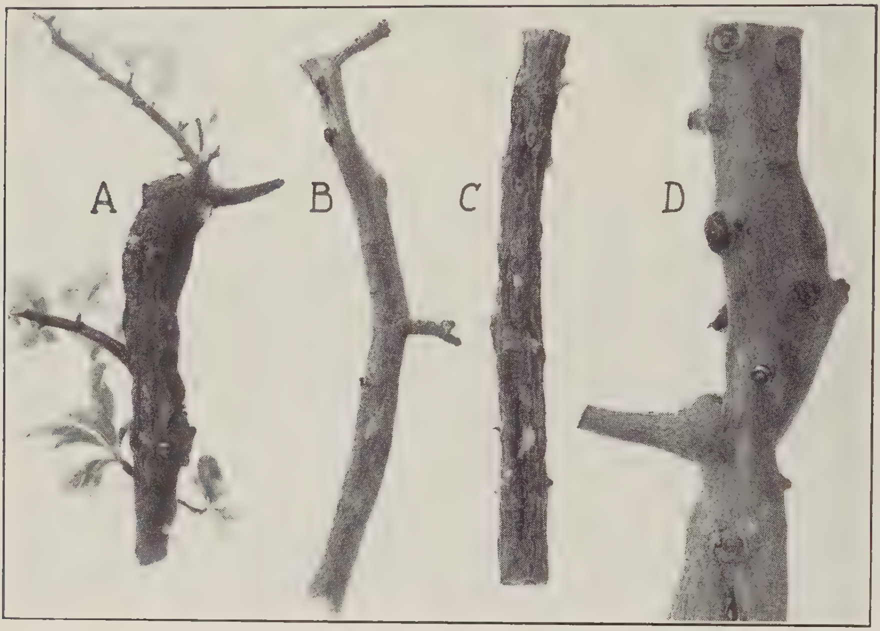

FIG. 1. A. Symptoms produced on *Pyracantha gibbsii yunnanensis* by graft inoculation from apple of the type shown at D. B. Depressions and distortion in Bosc pear associated with "measles" and fruit pitting. C. Pink Radiance rose naturally affected by rough bark condition. D. Gravenstein apple branch affected by "flat limb" or "crinkle-wood."

Other diseases have been seen on almond, apple, peach, and quince, which may well belong in this group; but the above may suffice to indicate that a very considerable number of diseases and kinds of woody plants are involved in the group.—H. EARL THOMAS, Division of Plant Pathology, University of California, Berkeley.

Two Genetic Characters of Tomato Fruits that Might be Mistaken for Symptoms of Disease.—Broad, dark-green stripes resembling mosaic symptoms were found in the peel of 2 tomato fruits on a plant of (T560) Michigan State Forcing (Ferry-Morse Seed Co.) at the Tomato Disease Laboratory, Jacksonville, Texas, in May, 1939. This abnormality apparently resulted from a mutation. In 1940, seed from these 2 fruits produced 23 plants with many striped fruits. Prominent, broad, dark-green stripes extended from stem to blossom end in the peel of the small green fruits. As the fruits developed to the green-wrap stage, deep pits and grooves formed in the peel of many of the striped fruits. On ripening, the dark-green stripes became yellow in contrast to the normal red part of the fruit (Fig. 1).

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[5] H. N. Hansen has collected *Alnus* (*rhombifolia?*) with cankers very similar to those of prune.

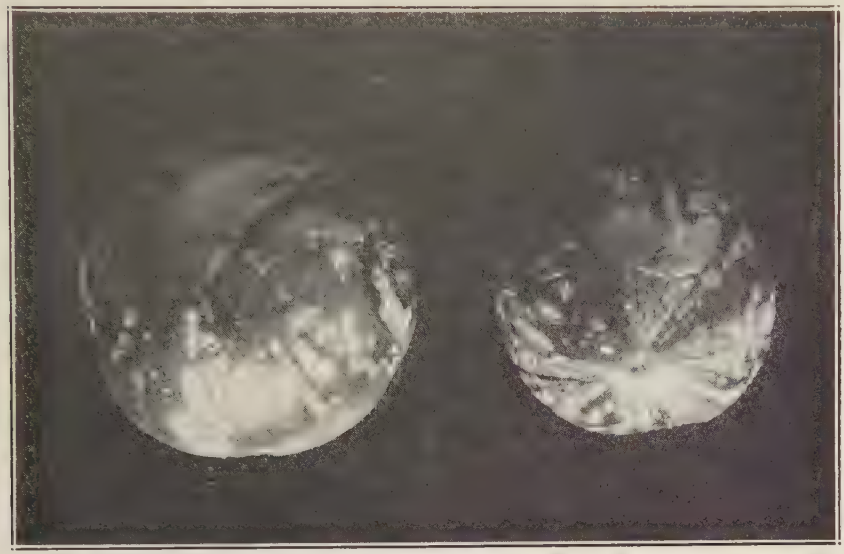

FIG. 1. Stripes, pits, and grooves in tomato-fruit peel of T560. × ½.

Seed from 4 of the striped fruits was tested for a third generation in fields in 1941. The parent fruits of T560B and T560D showed only stripes, while the parent fruits of T560C and T560E bore both stripes and pits. In the fields 72 per cent of the 184 plants of T560B and T560D bore striped and pitted fruits, while 94 per cent of the 221 plants of T560C and T560E produced striped and pitted fruits. These data are now interpreted as indicating inheritance of the stripe and pit characters. Many of the T560 tomato plants bore both striped and normal fruits, and the writer has observed such stripes and pits only in this selection.

None of these plants showed any other abnormality that might be associated with the stripes and pits, and there was no indication that this might be a virus disease. The stripes and pits were much more prominent in T560 than in fruit with tomato fruit pox,[1] which resembles mosaic and is not transmitted by seed. They differed from fruit stripe of tomato,[2] which causes raised light-green to ashy-gray stripes in the peel, associated with mild mosaic of the leaves.—P. A. YOUNG, Tomato Disease Laboratory, Texas Agricultural Experiment Station, Jacksonville, Texas.

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[1] Ivanoff, S. S. and P. A. Young. Tomato fruit pox. Phytopath. 30: 343–345. 1940.

[2] Jones, L. K. Fruit stripe of tomato caused by a tobacco type I virus. Phytopath. 30: 538–540. 1940.

Downy Mildew of Lima Beans in Colorado.—In 1889 Thaxter reported that the downy mildew was causing considerable damage to Lima beans in certain regions along the Atlantic Coast and described it as a new species, *Phytophthora phaseoli.* Since then it has been observed to be more or less common in many of the Eastern States and has been reported from as far west as Ohio.

It has been generally assumed that the downy mildew required high humidities for its development, which probably explains why it occurs mostly in the eastern part of the United States and the South, and less frequently in the interior. The fact that it appeared in northeastern Colorado during the summer of 1941 in an epidemic form suggests that either the arid regions sometimes become sufficiently humid for infection, or the downy mildew of Lima beans can adapt itself to arid conditions. During the many years the writers have visited this location each summer, downy mildew has never been previously observed.

Only a few varieties of Lima beans were grown in this locality where the observations were made. In one field each of the Henderson Bush, Woods Prolific, and Jackson Wonder varieties, 100 per cent of the plants were infected and the losses amounted to as much as 60–75 per cent. In one adjoining field the disease could not be found, showing it to be exceedingly spotty. Lima beans are grown entirely by surface irrigation in Colorado. The fungus occurred on the pods in all stages of their development and not on any other organs of the plant. The conidia, measuring somewhat larger than those described by Thaxter, were present in great abundance both on the outside and inside the pods. Zoospores and oogonia were not observed.

The summer of 1941, in Colorado, was unusual in that rains were rather frequent and the nights cool over a long period of time. Dews were frequent and abundant.

Lima bean seed is not grown in the States along the Atlantic Coast, where the disease is most prevalent, but only in Colorado and in the States farther west, whence downy mildew has not been reported, except in California. The disease has not been reported in California for many years, and its occurrence then was probably near the coast, where the humidity was high. In view of these facts it would be of unusual interest to know the source of the initial infection which reached epidemic proportions in Colorado in a single season.—L. L. HARTER AND W. J. ZAUMEYER, U. S. Horticultural Station, Beltsville, Md.

A New Virus Disease of Bean.—A virus disease of bean, tentatively designated as bean-mosaic virus 4, distinctly different from any hitherto described bean virus, was recently isolated from severely mottled pods of several varieties. Two types of symptoms, depending upon the bean variety inoculated, are produced. Local necrotic lesions are produced on some varieties, whereas on others only systemic mottled symptoms are produced.

In no case has any variety thus far tested exhibited both local and systemic infection on the same plant. Evidence shows only a single virus is involved.

The local lesions appear in about 2 to 4 days following inoculation. They are fairly circular, brownish red, and vary from 1 mm. to 3 mm. in diameter. The systemic mottled symptoms appear in about 10 days after inoculation and are not greatly unlike those produced by bean virus 1 on many varieties. In general, those varieties that are somewhat tolerant to bean virus 1 exhibit quite a severe mottling when infected with bean virus 4. The symptoms on the pods are more pronounced than those produced by any virus thus far reported to be infectious to bean. They are characterized by dark-green irregularly shaped, watersoaked-like blotches on some green-podded varieties and by greasy, shiny areas on others. On wax-podded varieties, greenish-yellow areas are noted. Infected pods may be slightly malformed, subnormal in length, and frequently curled at the end due to improper ovule development.

Seventy-nine varieties of beans have been inoculated and all have shown one symptom or the other after inoculation. Thirty-three varieties showed local lesion infection and 46 varieties were systemically infected. None of the varieties showing local lesions exhibited systemic infection, and *vice versa*. Of the 8 varieties known to be immune from bean virus 1, Corbett Refugee, Great Northern U.I. No. 59, and Red Mexican U.I. No. 34, exhibited local lesions when inoculated with bean virus 4. Idaho Refugee was heterozygous for the two symptoms, while U.S. No. 5 Refugee, Sensation Refugee No. 1066 and No. 1071, and Robust manifested mild systemic symptoms. Varieties showing local lesions upon artificial inoculation could be regarded as commercially resistant under field conditions. In tests thus far conducted, the host range of the virus is restricted to the Leguminoseae.

Preliminary studies on the properties of the virus show that the thermal inactivation point lies between 90° and 95° C., which is higher than that for any legume virus thus far described and approximates that of tobacco mosaic. It was infectious at a dilution of 1–500,000 and retained its infectivity after aging for 165 days at 18° C.

Immunological studies have shown no relationship to bean virus 1.

Little is known regarding the distribution of this virus under field conditions. It has been isolated from bean plants grown in Maryland, California, and Louisiana.—W. J. ZAUMEYER and L. L. HARTER, Division of Fruit and Vegetable Crops and Diseases, U. S. Horticultural Station, Beltsville, Md.

Classification and Nomenclature of the Pathogen Causing Bacterial Ring Rot of Potatoes.—In a recent article Thornberry[1] comments on the lack of consistency in citing the authorities for *Phytomonas sepedonica,* but the reason for this is not discussed. The main cause for the confusion appears to be the fact that Spieckermann first used the name *Bacterium sependonicum* in 1913 in a popular agricultural periodical[2] but omitted a description of the organism. The name was *nomen nudum* and, therefore, did not constitute true publication. It is this that certain investigators appear to have overlooked. In 1914 Spieckermann and Kotthoff[3] gave a description of the pathogen and other investigators accept the second article as the authentic publication of the species. The writers are of the same opinion. In 1920 E. F. Smith[4] referred to the species as *Aplanobacter sepedonica*, omitting a citation to authors. Further confusion arose in 1937 when Magrou[5] placed the pathogen in the genus *Phytomonas* only 3 months ahead of Savile and Racicot.[6]

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[1] Thornberry, H. H. Bacterial ring rot of potatoes in Illinois. Plant Dis. Rptr. 25: 509–510. 1941.

American investigators have followed Magrou in the use of the generic name *Phytomonas* for the pathogen, but no discussion has arisen as to the correct position of the species among the other bacteria. Elliott[7] has pointed out the invalidity of the name *Phytomonas;* and Burkholder[8] and Dowson[9] have shown that as the genus exists at present it is composed of a heterogeneous collection of bacteria. The existing species should be allocated to other genera. Jensen,[10] in discussing the genus *Corynebacterium,* states that possibly *Phyt. sepedonica* belongs here. This genus was established in 1896 by Lehmann and Neumann[11] for the diphtheria bacterium, and has been accepted by bacteriologists at large. A thorough study of the morphology and physiology of the ring-rot pathogen has convinced the writers that this organism has all the characteristics of a *Corynebacterium.* The most characteristic features placing it in this category are (1) its pleomorphism including large numbers of club-shape cells, (2) non-motility, (3) V- or L-shape cells indicating "snapping division," (4) its Gram reaction, non-acid fastness, and its uneven staining, and (5) its cells occurring mostly singly, very seldom even in pairs. Physiological properties characteristic of *Corynebacterium* are (1) its demand for a complex medium rich in protein, (2) its strict aerobic growth, (3) its general biochemical inactivity including poor gelatine liquefaction, poor starch hydrolysis, slow action on litmus milk, etc., and (4) its very slow rate of growth on all ordinary media.

On the basis of these major and minor characteristics it is proposed that

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[2] Spieckermann, A. Zur Kenntnis der in Deutschland auf treten den Gefasskrankheiten der Kartoffelplanze. Illus. Landw. Zeitung. **33**: 680–682. 1913.

[3] ——— and P. Kotthoff. Untersuchungen über die Kartoffelpflanze und ihre Krankheiten. I. Die Bacterienringfäule der Kartoffelpflanze. Landw. Jahrb. **46**: 659–732. 1914.

[4] Smith, E. F. Bacterial diseases of plants. p. 207. Saunders, (Philadelphia). 1920.

[5] Magrou, J. *Phytomonas. In* Dictionnaire de bactéeries pathogènes by P. Hauduroy, G. Ehringer, A. Urbain, G. Guillot and J. Magrou. Masson et Cie (Paris). 326–437. 1937.

[6] Savile, D. B. O. and H. N. Racicot. Bacterial wilt and rot of potatoes. Sci. Agr. **17**: 518–522. 1937.

[7] Elliott, Charlotte. The genus *Phytomonas.* Phytopath. **27**: 1181–1182. 1937.

[8] Burkholder, W. H. The taxonomy and nomenclature of the phytopathogenic bacteria. Phytopath. **29**: 128–136. 1939.

[9] Dowson, W. J. On the systematic position and generic names of the Gram-negative bacterial plant pathogens. Zentralb. Bakt. Abt. II. **100**: 177–193. 1939.

[10] Jensen, H. L. Studies on saprophytic Mycobacteria and Corynebacteria. Proc. Linn. Soc. New S. Wales. **59**: 19–62. 1934.

[11] Lehmann, K. B., and R. Neumann. *Corynebacterium.* Atlas und Grundriss der Bakteriologie. Teil. II. 1 Auft. 108 and 350. Lehmann (München). 1896.

the name of the ring-rot organism be changed to **Corynebacterium sepedonicum** (Spieckermann et Kotthoff) comb. nov.—J. B. SKAPTASON and W. H. BURKHOLDER, Department of Plant Pathology, Cornell University, Ithaca, New York.

Scolytus sulcatus and Apple Trees in Relation to the Dutch Elm Disease Control Program.—*Scolytus sulcatus* LeC., a bark beetle, commonly reported on apple and occasionally on related species of trees in central and eastern New York, western Connecticut, and northern New Jersey, has been reported feeding on and breeding in elm.[1] Recently, *S. sulcatus* has been found capable of transmitting *Ceratostomella ulmi* Buisman under controlled conditions from infected elm logs to nursery elms in cages.[2]

A preliminary investigation of the pathogenicity and longevity of *Ceratostomella ulmi* in apple trees and wood has been conducted during the past 2 years. On June 5, 1939, 100 wild-apple seedlings (*Pyrus malus* L.), 1 to 1½ in. in diameter at the base and 5 to 12 ft. tall, were inoculated at one point near the base of the trunk. Inoculation was effected by making a vertical tangential incision into the xylem with a ⅛-in. wood chisel and then immediately filling the wound with an aqueous suspension of spores of *C. ulmi.* Ten comparable trees were similarly inoculated with sterile water as checks. No external symptoms of the disease were noted on any of the trees. During the first week of September, 1939, 50 of the inoculated trees and 5 of the check trees were cut and examined for infection. Light-brown discoloration, which in 16 cases extended into the small twigs, was observed in the xylem of all of the 50 inoculated trees (Fig. 1). The minimum, maximum, and average spreads of *C. ulmi* from the points of inoculation, as determined by the recovery of *C. ulmi* by culturing, were 5.25, 111.68, and 47.75 inches, respectively. *C. ulmi* could not be isolated from any of the check trees.

After examination the trunks of the 50 inoculated trees were placed outdoors on the ground in dense shade. On September 24, 1939, coremia were noted growing on cut surfaces of 11 of the trunks. The heads from several coremia on each trunk were cultured and in each case proved to be *Ceratostomella ulmi.* On October 1–3, 1940, cultures were made of discolored streaks in the xylem from the 50 trunks that had been stored on the ground approximately 13 months. *C. ulmi* was recovered from 19 of the trunks. These 19 trunks were cultured again on March 5, 1941, but *C. ulmi* was recovered in only 2 cases. Efforts to recover *C. ulmi* from these remaining 2 trunks on September 12, 1941, were unsuccessful, the organism apparently having died out sometime between the 18th and 24th month of storage.

On September 23, 1941, 27 months after inoculation, samples were cut from 10 of the inoculated trees remaining. *Ceratostomella ulmi* was recovered from 9 of the 10 trees.

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[1] Pechuman, L. L. A preliminary study of the biology of *Scolytus sulcatus* LeC., Jour. Econ. Ent. **31**: 537–543. 1938.

[2] Buchanan, W. D. *Scolytus sulcatus* LeC. transmits Dutch elm disease fungus under controlled conditions. Jour. Econ. Ent. **33**: 250–251. 1940.

In December, 1940, and in January, 1941, 107 samples of apple wood infested with *Scolytus sulcatus* were collected at 17 points in 4 counties in New Jersey and at 15 points in 3 counties in New York within 1,000 feet of where elms infected with *Ceratostomella ulmi* had been found and removed by the Bureau of Entomology and Plant Quarantine.[3] Attempts to isolate *C. ulmi* from representative maternal galleries from the 107 collections were made by the method described by J. M. Walter.[4] Of 535 none yielded *C. ulmi*.

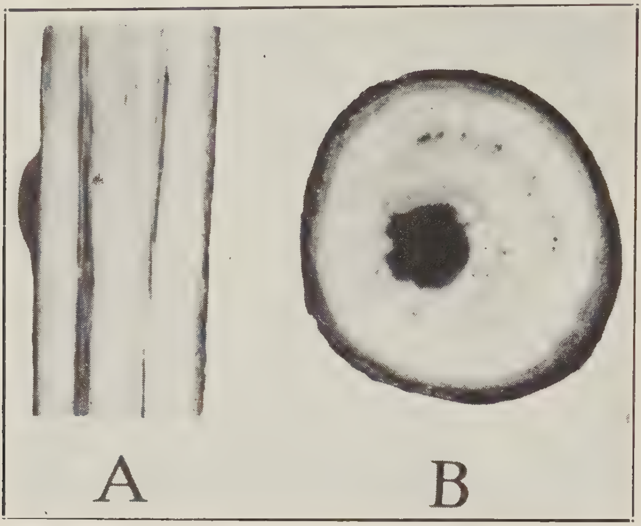

FIG. 1.—(A) Longitudinal section and (B) cross section of apple branch showing streaks of discoloration resulting from inoculation with *Ceratostomella ulmi*.

The remaining infested material was then placed in rearing jars in the rearing room of the Division of Forest Insect Investigations, Morristown, N. J. The adult *Scolytus sulcatus* were collected regularly as they emerged, and were cultured. None of the 14,311 beetles from the infested material yielded *Ceratostomella ulmi*.

Even though *Ceratostomella ulmi* is capable of remaining viable for considerable periods of time in living apple trees and also in apple wood, the probability of *Scolytus sulcatus* transmitting the fungus to apple wood in nature and of the apple wood being an important source of inoculum for later spread, apparently is relatively unimportant at present in the Dutch elm disease control program.—S. J. SMUCKER, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, Morristown, New Jersey.

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[3] Acknowledgment is hereby made of the assistance rendered by J. F. Wootten and the various County Supervisors of the Dutch Elm Disease Control Office of the Bureau of Entomology and Plant Quarantine in making these collections possible. Acknowledgment is also made of the assistance rendered by W. D. Buchanan and Charles Griswold of the Division of Forest Insect Investigations in collecting apple wood infested with *Scolytus sulcatus,* and in rearing *S. sulcatus* from the infested material.

[4] Walter, J. M. Technique advantageous for the isolation of *Ceratostomella ulmi* from bark beetles. Phytopath. (Abstracts) **25**: 37–38. 1935.

BOOK REVIEW

THE AMERICAN SOCIETY OF AGRONOMY AND THE NATIONAL FERTILIZER ASSOCIATION. *Hunger Signs in Crops*, 340 pp., 95 figs., with 79 colored plates. $2.50. Judd & Detweiler and Standard Engraving Co. (Washington). 1941.

The publication of ''Hunger Signs in Crops'' makes available to plant pathologists a valuable reference for use in diagnosing plant diseases.

The book presents up-to-date information on the abnormal macroscopic conditions that are induced in agricultural plants when the supply of one, or more, of the food materials in the soil is insufficient. Since a deficiency of any one element may induce quite different symptoms on different plants, the plants are for the most part dealt with individually. Thus, there are chapters covering nutrient deficiencies in tobacco, corn and small grains, potato, cotton, truck crop plants, deciduous fruits, legumes, and citrus. The food materials dealt with are nitrogen, phosphorus, potassium, magnesium, calcium, boron, manganese, sulphur, iron, copper, and zinc. Each chapter has been written by specialists who have made extensive investigations on the crop concerned. Consequently, the description of the various deficiency symptoms are characterized by clarity of definition and a full appreciation of the complexities involved. At the end of each chapter a selected list of references is given. An outstanding value of the book is the large number of photographs in natural color illustrating the various disorders described in the text. There are 79 full-page color plates and many photographs in black and white. It is a text that should be on the shelf of every plant pathologist who contacts field problems, and who must make field diagnoses.—H. R. MCLARTY, Dominion Laboratory of Plant Pathology, Summerland, British Columbia.

NOTICES

SUMMER MEETING AMERICAN PHYTOPATHOLOGICAL SOCIETY

The Summer Meeting of the American Phytopathological Society will be held June 25–26, 1942, in the Secor Hotel, Toledo, Ohio. The first session will begin at 10:00 a.m., June 25.

The purpose of this meeting is to assist in coordinating the various war emergency efforts of the Society. The functions, accomplishments, and future plans of the Society's War Emergency Committee, subcommittees, and sectional committees will be reported briefly with plenty of opportunity for discussion. Topics to be discussed, if time permits, include: fungicides —their availability and substitutes, coordinated research programs, duties of extension pathologists, draft deferment, disease surveys, quarantines, seed treatment and certification, cooperation with workers in other sciences, and other timely subjects.

A summer meeting of this nature is new for our Society, but presents an excellent opportunity to become intimately acquainted with the emergency programs and problems, and to contribute constructive criticisms and suggestions for future procedure.

When making hotel reservations indicate that you expect to attend the American Phytopathological Society Meetings.

COMMITTEE ON PROGRAM AND ARRANGEMENTS
1942 SUMMER MEETING

CONSERVATION OF SCHOLARLY JOURNALS

The American Library Association created this last year the Committee on Aid to Libraries in War Areas, headed by John R. Russell, the Librarian of the University of Rochester. The Committee is faced with numerous serious problems and hopes that American scholars and scientists will be of considerable aid in the solution of one of these problems.

One of the most difficult tasks in library reconstruction after the first World War was that of completing foreign institutional sets of American scholarly, scientific, and technical periodicals. The attempt to avoid a duplication of that situation is now the concern of the Committee.

Many sets of journals will be broken by the financial inability of the institutions to renew subscriptions. As far as possible they will be completed from a stock of periodicals being purchased by the Committee. Many more will have been broken through mail difficulties and loss of shipments, while still other sets will have disappeared in the destruction of libraries. The size of the eventual demand is impossible to estimate, but requests received by the Committee already give evidence that it will be enormous.

With an imminent paper shortage attempts are being made to collect old periodicals for pulp. Fearing this possible reduction in the already limited supply of scholarly and scientific journals, the Committee hopes to enlist the cooperation of subscribers to this journal in preventing the sacrifice of this type of material to the pulp demand. It is scarcely necessary to mention the appreciation of foreign institutions and scholars for this activity.

Questions concerning the project or concerning the value of particular periodicals to the project should be directed to Wayne M. Hartwell, Executive Assistant to the Committee on Aid to Libraries in War Areas, Rush Rhees Library, University of Rochester, Rochester, New York.

ANNOUNCEMENT

"In the July issue of *The Botanical Review* there will be an extensive review by Dr. W. J. Robbins and Dr. V. Kavanagh of the New York Botanical Garden and Columbia University on 'Vitamin Deficiencies of the Filamentous Fungi' which will be a tabulated summary of growth substance requirements of 375 species. Authors' reprints will not be distributed, but separate copies may be purchased for 50 cents each by pre-publication orders to *The Botanical Review,* New York Botanical Garden."

THE report of the Committee on Nomenclature and Classification of Plant Viruses having been, through error, omitted from the Annual Report of the Society, published in the April number of PHYTOPATHOLOGY, is here presented, together with the covering motion adopted by the Society.

The motion made, seconded, and adopted by the Council in session at Dallas, Texas, Dec. 28, 1941, and subsequently presented to the Society in regular business session, was, in substance as follows: The Council realizes that this committee has a very difficult assignment, and that it has been a hard-working committee, and, in view of the fact that it has not yet been possible to arrive at final recommendations, the Council recommends that the present reports be received with appreciation as reports of progress, and laid on the table. Following is the report of the committee:

REPORT OF THE COMMITTEE ON NOMENCLATURE AND CLASSIFICATION OF PLANT VIRUSES

The Committee approves (6 for; Gardner and Johnson against) the designation of plant viruses by a system of Latin binomials such as that used for scientific naming of organisms. An essentially similar system is used in naming the substances dealt with in Materia Medica. There is good ground for hope that the Committee will soon sponsor and recommend to the Society for approval and publication a simplified, compromise system of virus classification.

During the past year much progress has been made by some of the subcommittees selected to compile the synonyms and descriptions of viruses associated with the plants of the principal crop divisions. Completion of the assignments will be hastened if the use of a Latin binomial as the preferred designation for a virus is generally accepted.

EUBANKS CARSNER, Chairman	JAMES JOHNSON
C. W. BENNETT	H. H. MCKINNEY
M. W. GARDNER	H. H. THORNBERRY
F. O. HOLMES	FREEMAN WEISS

ORMOND ROURKE BUTLER
1877-1940

I. E. MELHUS

Dr. Ormond Rourke Butler was head of the Department of Botany of the University of New Hampshire and Botanist of the New Hampshire Agricultural Experiment Station from 1912 until the time of his death.

The copper fungicides received Dr. Butler's best efforts for many years, and his contributions bear evidence of his originality and thoroughness. It was natural that his researches on the properties of fungicides should lead him to give special attention to the control of apple and potato diseases. In all his research he worked carefully, feeling that no price was too great to pay for accuracy. He thoroughly understood his field and had outstanding ability in organizing his research. Dr. Butler read the literature to such an extent that it became a part of him. He refused to be drawn into any argument and enjoyed a clear exposition of another person's point of view. Favor and prejudice never were permitted to influence his findings in research. All scientific data obtained through his investigations could be accepted without any question as to their authenticity.

Although he seldom was ill, he was not robust. Throughout his career as a scientist, however, he worked tirelessly. He did not have a wide circle of friends but rather a few very loyal ones. His sterling character was manifested by his admiration and respect for his father and mother and his loyalty to his friends. He was generous, sympathetic, and retiring almost to his own detriment.

Dr. Butler had several hobbies. He was greatly interested in gardening, particularly in the growing of rhododendrons. Another hobby was that of cooking, which began with a study that he made on the cooking qualities of potatoes. It is reported that he left three notebooks filled with data and recipes.

Symphonic music, the ballet, and opera were among his diversions. He had a thorough command of the French language and prized his collection of French books. It was always his dream that he might return to visit in France and Switzerland, where he spent most of his youth.

Ormond Rourke Butler was born in Melbourne, Australia, on August 14, 1877. He was the elder of two sons of Thomas and Mary Anne (Rourke) Butler. His mother was the daughter of Henry Rourke, a contractor and cattleman in Australia. In 1895 he received the diploma of the Institute Nationale Agricole, Lausanne, Switzerland, where he completed the equivalent of our grammar and high school studies. That same year he came to the United States and was granted citizenship fourteen years later. From 1895 to 1900 he was viticulturist-horticulturist in California. He entered the University of California where he received the degree of Bachelor of

Science and, following this, the degree of Master of Science, in 1905. From 1906 through 1908 he was assistant at the Southern California Pathological Laboratory at Whittier, California. Thereafter he pursued graduate work at Cornell University, where he received the degree of Doctor of Philosophy in 1910. He then became research instructor in the Department of Horticulture at the University of Wisconsin. From 1912 until the time of his death he was head of the Department of Botany of the University of New Hampshire and Botanist of the New Hampshire Agricultural Experiment Station. Dr. Butler died on October 24, 1940. He is survived by two sons, Robert E. and Alexander R. Butler. His wife, the former Roberta Van Horn, a graduate of North Dakota State College, preceded him in death on March 8, 1929.

ORMOND ROURKE BUTLER
(1877–1940)

Dr. Butler was a charter member of The American Phytopathological Society, a member of the American Society of Agronomy, a Fellow of the American Association for the Advancement of Science, and a member of Sigma Xi and Phi Kappa Phi.

At the time of his death Dr. Butler was engaged in several experiment station research projects, one of which was "Control of Apple Scab." His data and observations on this problem are now being prepared for publication. Other investigations included effect of temperature and other environmental factors on the expression of symptoms of potato mosaic, spray injury by lime-sulphur, effect of temperature on potatoes in storage, control of bitter pit of apple, peony diseases, and the control of poison ivy.

LIST OF PUBLISHED PAPERS

1. Observations of some vine diseases in Sonoma County, California. California Agr. Exp. Stat. Bull. 168. 1905.
2. Gum disease of citrus trees in California. California Agr. Exp. Stat. Bull. 200. 1908. (Co-author with Ralph E. Smith.)
3. Observations on the California vine disease. Mem. Tor. Bot. Club **14**: 111–153. 1910.
4. A study of gummosis of Prunus and Citrus, with observations on squamosis and exanthema of Citrus. Ann. of Bot. 25: 107–153. 1911.
5. A note on the significance of sugar in the tubers of *Solanum tuberosum*. Bull. Tor. Bot. Club **40**: 110–118. 1913.
6. Studies on the factors affecting the culinary quality of potatoes. Jour. Amer. Soc. Agron. **5**: 1–33. 1913. (Co-author with F. B. Morrison and F. E. Boll.)
7. Bordeaux mixture. I. Physico-chemical studies. Phytopath. **4**: 125–180. 1914.
8. Notes on the preparation of Bordeaux mixture. New Hampshire Agr. Exp. Stat. Circ. 15. 1914.
9. Bordeaux mixture. New Hampshire Agr. Exp. Sta. Tech. Bull. 8. 1914.
10. Methods of preparation and relative value of Bordeaux mixture. Off. Rept. Sess. Internat. Cong. Vit., pp. 151–160. 1915.
11. The influence of temperature on decomposition in Bordeaux mixture. Prog. Agr. et Vit. (Ed. l'Est-Centre) **36**: 15–18. 1915.
12. The cuprammonium washes, their preparation, biological properties and application. Phytopath. **7**: 235–268. 1917.
13. On the cause of the alternate bearing in the apple. Bull. Tor. Bot. Club **44**: 85–96. 1917.
14. Physiology of the apple. New Hampshire Agr. Exp. Stat. Tech. Bull. 13. 1917. (Co-author with T. O. Smith and B. C. Curry.)
15. How to control the snapdragon rust. Florists' Ex. **43**: 353. 1917.
16. On the preservation of phytopathological specimens in their natural colors. Phytopath. **8**: 66–68. 1918.
17. Scorching due to cuprammonium washes. Prog. Agr. et Vit. (Ed. l'Est-Centre) **39**: 104–107. 1918.
18. The effect of the environment on the loss of weight and germination of seed potatoes during storage. Jour. Amer. Soc. Agron. **11**: 114–118. 1919.
19. Relative adhesiveness of the copper fungicides. Phytopath. **9**: 431–444. 1919. (Co-author with T. O. Smith.)
20. Field control of the snapdragon rust. Florists' Ex. **48**: 951. 1919.
21. Effect of wounds on loss of weight of potatoes. Jour. Amer. Soc. Agron. **11**: 304–305. 1919.
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THE INHERITANCE OF RESISTANCE TO USTILAGO NUDA[1]

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INTRODUCTION

Flower-infecting loose smut, caused by *Ustilago nuda* (Jens.) K. and S., is one of the most widely recognized diseases of barley in Missouri. The modified hot-water treatment (1) for destroying the dormant mycelium of *U. nuda* within the barley seed is difficult to apply, and, even under carefully controlled conditions, may cause some damage. It is desirable, therefore, to have available varieties that are resistant to the attack of this fungus and adapted to Missouri conditions. During the course of the breeding program to develop an adapted variety of winter barley resistant to *U. nuda*, an attempt was made to determine the behavior of the inheritance of resistance to this smut in various barley hybrids, and the linkage relations between the factors for resistance, hoods, six-row heads, and winter habit.

Zeiner (10) studied 8 crosses between barley varieties showing various degrees of susceptibility to *Ustilago nuda*. Resistance appeared to be dominant and controlled by a single factor, although the evidence was inconclusive. When two susceptible varieties were crossed there was no indication of transgressive segregation toward greater resistance.

Nahmmacher (5) confirmed many of Zeiner's results; the uncertainty of the infection method, however, made it difficult to obtain an accurate analysis of the factors governing the reaction of the F_3 progenies.

The low percentage of smutted heads resulting from artificial inoculation with chlamydospores has been one of the greatest handicaps in varietal testing for resistance to *Ustilago nuda*. Methods involving the clipping or spreading of the glumes have lowered the vitality of the seed (8, 10). Tapke (8) and Moore (4) have developed methods that give fairly good infection with this loose smut of barley and ordinarily produce plump and viable seed from inoculated flowers.

MATERIALS AND METHODS

In comparative tests the partial-vacuum method of inoculation described by Moore (4) gave the most satisfactory results and was used throughout this investigation. The flowers at the 3 terminal and basal joints of the rachis were removed and the remainder of the head inoculated from 12 to 24 hours after anthesis of the terminal flowers. The vacuum was supplied by making 20 strokes with an automobile pump having a $1\frac{3}{4}$-inch bore.

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[1] The subject matter of this paper was submitted to the faculty of the Graduate College of the University of Missouri in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The writer wishes to express his appreciation to Dr. C. M. Tucker for his supervision and criticism during the course of this investigation and for his suggestions in the preparation of the manuscript.

Inoculated plants were placed for 72 hours, in an incubation chamber, in the greenhouse with a humidity range of 85 to 95 per cent and a temperature range of 15 to 30° C. Night temperatures usually were maintained between 15° and 18°, and, during the daytime, between 20° and 22° C., except in late spring, when they frequently reached 30° C. Microscopic examination of the stigmas showed spore germination under these conditions in less than 24 hours.

The inoculum supply was built up and maintained on Missouri Early Beardless winter barley from a single infected head of the same variety. Spore structure and type of germination proved it to be *Ustilago nuda.* Mature smutted heads were collected and stored, in a refrigerator, from 30 to 60 days at 3° to 5° C. Germination of the spores was always tested before applying them, and only samples with a germination of 50 per cent or higher were used. The spore suspension used as inoculum consisted of approximately 50 mg. of chlamydospores of *U. nuda* in 60 ml. of tap water.

Inoculated seed was germinated in the laboratory and planted in paper plant bands, where they were kept until they were approximately 3 inches high, and then were transplanted to the field. This procedure reduced the seedling mortality loss of infected material.

Data on the susceptibility of the parent varieties to the Missouri Early Beardless collection of *Ustilago nuda* are presented in table 1. There was a fairly large variation in the limits of susceptibility, even though care was taken to maintain uniform inoculation and incubation conditions. Total infection in the susceptible varieties was not obtained. This may be due to differing varietal susceptibility or to some fault in the inoculation method.

TABLE 1.—*Susceptibility of barley varieties to the Missouri Early Beardless collection of Ustilago nuda when inoculated by the partial-vacuum method*

Variety	Tests	Seeds planted	Plants obtained	Plants infected	Average infection[a]
	Number	*Number*	*Number*	*Number*	*Per cent*
Missouri Early Beardless	8	858	754	399	52.92 ± 7.61
Colsess IV	8	563	504	381	75.59 ± 12.39
Trebi	9	649	514	7	1.36 ± 0.607
Hordeum deficiens	5	521	477	5	1.05 ± 0.246

[a] Standard errors.

INFECTION OF HYBRID PROGENIES

In order to expose the progeny of a hybrid barley plant to infection by *Ustilago nuda,* the heads were inoculated at flowering time. The tissues through which the infecting hyphae penetrate to the ovary are those of the parent plant, which may differ genetically from those of the embryo to be tested for susceptibility. It was necessary, therefore, to ascertain whether the results of inoculation were influenced by the resistance or susceptibility of the parent-plant tissue or by the resistance or susceptibility of the embryo. For example, when the flowers of F_1 hybrids were inoculated, was the appearance of smut in the F_2 plants dependent on the ability of the fungus to penetrate the floral tissues of the F_1 hybrid, or on its ability to infect the tissues of the F_2 embryo? An examination of the F_1 hybrids of reciprocal crosses between resistant and susceptible varieties should yield information on this point. The F_1 generation was tested by inoculating the flowers of the female parent 24 hours after the introduction of pollen from the male parent. Unfortunately, F_1 material of only one series of reciprocal crosses was available.

Crosses between Trebi (1.36 per cent susceptible) and Colsess IV (75.59 per cent susceptible), in which Trebi was used as the female parent (Table 2), yielded 57 F_1 plants, of which 3.51 per cent were infected. The reciprocal cross yielded 48 F_1 plants, of which 6.25 per cent were infected. Although the percentage of infection was considerably higher when the susceptible female parent was inoculated, the difference is not significant.

A further comparison of the effect of the reaction of the parent on embryo infection may be drawn from the results of inoculation of the F_1 hybrids, Trebi × Missouri Early Beardless and Missouri Early Beardless × *Hordeum deficiens*. When Trebi (1.36 per cent susceptible) was pollinated with pollen of Missouri Early Beardless (52.92 per cent susceptible) and inoculated, 84 F_1 plants were secured, of which 1.19 per cent were infected. When stigmas of Missouri Early Beardless (52.92 per cent susceptible) were pollinated with pollen of *H. deficiens* (1.05 per cent susceptible) and inoculated, all of the 36 F_1 hybrids showed no infection. While the data are not extensive, there are clear indications that the infection of the hybrids is determined by the nature of the embryo, rather than by the resistance or the susceptibility of the floral tissues of the female parent on which they are borne.

The hypothesis that stigmatic tissues are susceptible to invasion by infection hyphae is further supported by the behavior of the F_2 hybrids. When the stigmas of F_1 hybrids are inoculated, the infection hyphae must penetrate the floral tissues bearing the F_1 complement of genes for resistance and susceptibility. That such tissues are susceptible is proved by the increased percentages of infection among F_2 plants (Table 3).

Similar results were obtained by Larose and Vanderwalle (2) and Milan (3) with wheat hybrids inoculated with *Ustilago tritici* (Pers.) Rostr. They inoculated plants of the F_1 generation of reciprocal crosses and found resistance dominant, regardless of whether a resistant or a susceptible plant was used as the female parent.

INHERITANCE OF RESISTANCE FACTORS

The study of the method of inheritance of resistance to *Ustilago nuda* was complicated by the fact that it was not possible to obtain 100 per cent infection, even in Colsess IV, the most susceptible variety tested (Table 1). Since the breeding program was directed primarily toward incorporating desirable agronomic characters with smut resistance, many of the crosses involved the use of Missouri Early Beardless, which showed an average infection of about 50 per cent by the most effective inoculation methods available. It was considered desirable, therefore, to observe the behavior of inoculated F_1, F_2, and F_3 progenies.

F_1 Generation

The data for the infection of F_1 barley hybrids inoculated with chlamydospores of *Ustilago nuda* (Table 2) suggest that the resistance of Trebi and *Hordeum deficiens* is transmitted as a dominant character. Since neither variety is immune, an occasional smutted plant is to be expected among the F_1 hybrids. In the reciprocal crosses of Trebi with Colsess IV, however, the percentage of infection of inoculated F_1 hybrids was somewhat greater than that obtained by inoculation of Trebi, indicating that the resistance of Trebi may not be completely dominant.

TABLE 2.—*Infection of F_1 barley hybrids inoculated with chlamydospores of Ustilago nuda*

Hybrid	Total plants	Infected plants	
	Number	*Number*	*Per cent*
Colsess IV × Trebi	48	3	6.25
Trebi × Colsess IV	57	2	3.51
Trebi × Missouri Early Beardless	84	1	1.19
Missouri Early Beardless × *Hordeum deficiens*	36	0	0.0

F_2 Generation

Infection data for this generation are shown in table 3. The proportion of infected plants in the reciprocal crosses of Colsess IV with Trebi suggests a dihybrid ratio. It must be borne in mind, however, that the susceptible parent did not develop 100 per cent infection when inoculations were made at different periods, even though care was taken to maintain uniform conditions for inoculation and incubation. It, therefore, appears unlikely that resistance is dependent on the occurrence of two dominant factors, either of which might confer resistance on the progeny.

When Missouri Early Beardless was crossed with either Trebi or *Hordeum deficiens*, a very low percentage of infection was obtained in the second generation as compared with that obtained when Trebi was crossed with Colsess IV. Since the variety Trebi was used in crosses with both Missouri Early Beardless and Colsess IV, there is an indication that the difference in susceptibility of Missouri Early Beardless and Colsess IV (Table 1) is apparent also in the hybrid progenies from crosses involving those two varieties.

The large percentage of resistant plants in the crosses of Missouri Early Beardless with Trebi and with *Hordeum deficiens* indicates that the inheritance of resistance to *Ustilago nuda* may be controlled by several genetic factors. Since the crosses of Trebi and Colsess IV indicate the probable existence of a single factor for resistance in Trebi, it seems possible that a factor for resistance also may have been introduced into the Trebi × Missouri Early Beardless cross by the Missouri Early Beardless parent. Crosses between *Hordeum deficiens* and Missouri Early Beardless reacted similarly to the Trebi × Missouri Early Beardless cross.

TABLE 3.—*Infection of F_2 barley hybrids inoculated with chlamydospores of Ustilago nuda*

Hybrid	Seeds planted	Plants	Infected plants	
	Number	*Number*	*Number*	*Per cent*
Colsess IV × Trebi	142	112	12	10.71
Trebi × Colsess IV	216	212	16	7.54
Total	358	324	28	8.64
Missouri Early Beardless × *Hordeum deficiens*	109	97	3	3.09
Hordeum deficiens × Missouri Early Beardless	211	186	8	4.30
Total	320	283	11	3.88
Trebi × Missouri Early Beardless	234	191	4	2.09
Colsess IV × Missouri Early Beardless	133	117	69	58.97

In view of the apparent difference in reaction of the hybrid progenies when the two susceptible varieties, Colsess IV and Missouri Early Beardless, were used with the same resistant variety, it was of interest to observe the infection obtained in inoculated F_2 hybrids from the cross, Colsess IV × Missouri Early Beardless. Of a total of 117 inoculated F_2 plants, 58.97 per cent were infected (Table 3). This more nearly approaches the average percentage of infection obtained by inoculating Missouri Early Beardless instead of Colsess IV, and may indicate that Missouri Early Beardless possesses a weak factor for resistance that is absent in Colsess IV.

F_3 Generation

The F_3 progenies were classified as either resistant, segregating, or susceptible. The separation of the segregating and susceptible progenies was more or less arbitrary. Due to variation in effectiveness of inoculation and to the failure to obtain 100 per cent infection of the susceptible parent, it was difficult to separate the segregating and susceptible classes. Since the lowest percentage of infection obtained by inoculation of Colsess IV was 58.63, progenies in which the percentage of infected individuals was more than 55 per cent were arbitrarily classified as susceptible; the segregating progenies included those in which the percentage of infected individuals was 1 to 55; progenies free from infection were considered resistant. The lowest percentage of infection obtained by inoculation of Missouri Early Beardless with *Ustilago nuda* was 35.90; therefore, 35 per cent was chosen as the separation point between segregating and susceptible progenies when Missouri Early Beardless was used as the susceptible parent. Some erroneous classifications probably were made, particularly among progenies containing small numbers of plants. The average number of plants in the F_3 progenies was 19.3. Progenies containing less than 12 plants were not included.

A total of 122 families inoculated with *Ustilago nuda* were grown from a smut-free F_2 generation of the cross Colsess IV × Trebi (Table 4). There were 56 F_3 families with 887 individuals containing no smutted plants, while 66 families containing 955 plants showed infection ranging from 7.69 to 100 per cent. Only 6 of these families had less than 25 per cent infection, while 4 had 100 per cent. A total of 31 families (25.41 per cent) contained over 55 per cent of infected plants, which is within the limits of susceptibility of the susceptible parent. The greater number of infected F_3 populations was fairly well distributed between 20 and 75 per cent, with some tendency toward the formation of two groups, one between 20 and 40 per cent, and the other between 50 and 75 per cent.

TABLE 4.—*Infection of F_3 families from non-inoculated plants when inoculated with chlamydospores of Ustilago nuda*

Hybrid	Prog.	Seeds pltd.	F_3 plants	F_3 families in infection percentage classes		
				0.0	1–54.99	55–100
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Colsess IV × Trebi	122	2357	1842	56	35	31
Trebi × Colsess IV	62	1150	939	22	21	19
Total	184	3507	2781	78	56	50

The reciprocal cross, Trebi × Colsess IV, reacted similarly (Table 4). The number of susceptible progenies in both crosses approaches that expected if resistance is controlled by a single genetic factor. When the data from the reciprocal crosses are combined, 184 F_3 progenies are distributed in 5 per cent groups (Fig. 1). The percentage of families that reacted in a manner characteristic of the susceptible parent suggests that the inheritance of resistance is dependent on a single factor. The preponderance of resistant progenies and the small number of segregating progenies may be due to the small numbers of plants in the families, or to variation in effectiveness of the inoculation methods, and failure to secure 100 per cent infection in the susceptible parent, or, yet again, to partial failure of germination. Thren (9) reported that introducing a large number of viable spores into a flower reduced its ability to produce viable seed. He found that the loss, 21.4 per cent of the inoculated seeds as compared with 7 per cent of the controls, was due primarily to failure of the seeds to germinate, or, to weak germination followed by death of the seedlings.

Like results were obtained in this investigation. A larger percentage

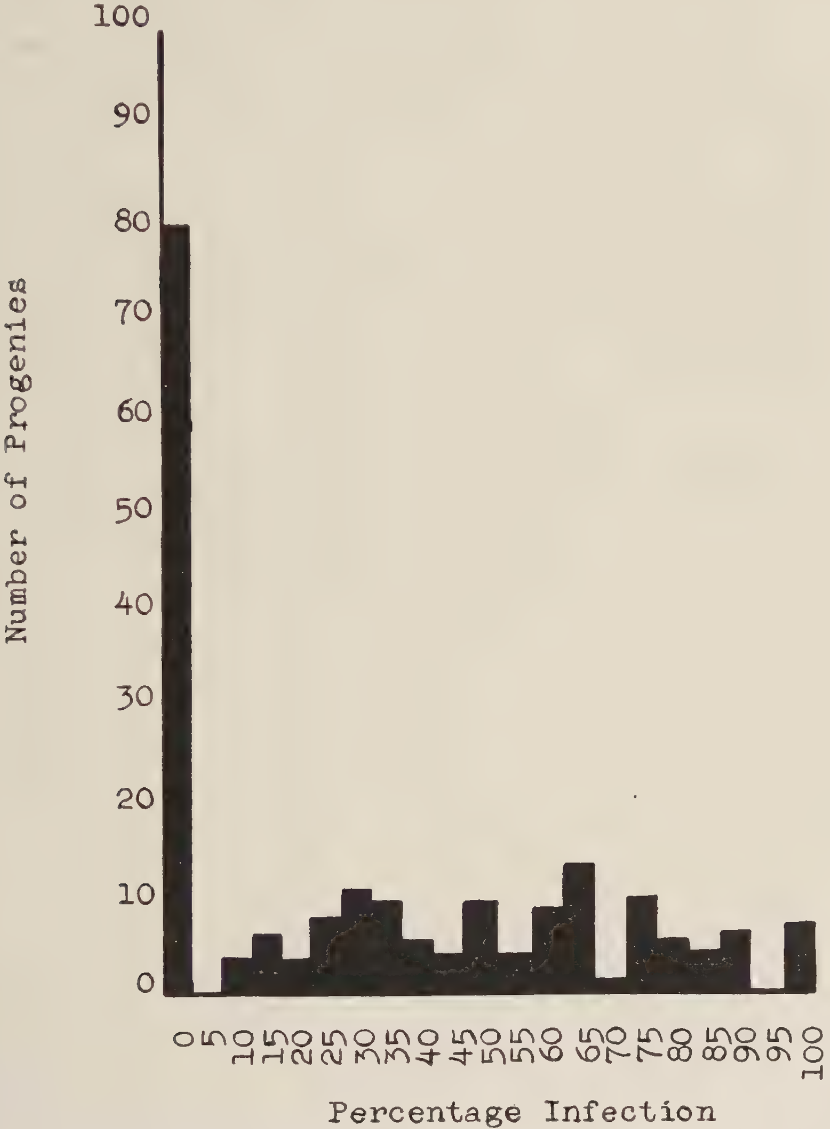

FIG. 1. Distribution of F_3 progenies in 5 per cent infection classes in reciprocal crosses of Trebi with Colsess IV when artificially inoculated with chlamydospores of *Ustilago nuda*.

of seeds in the F_3 families containing infected plants failed to germinate than in F_3 families with no infection (Table 5), indicating that the penetration of the embryo of the barley flower by the mycelium of *Ustilago nuda* has a detrimental effect on the development and maturation of the seed,

TABLE 5.—*Percentages of non-viable seeds in infected and uninfected F_3 families following inoculation with chlamydospores of Ustilago nuda*

Hybrid	Seeds planted		F_3 plts. obtained		Non-viable seeds	
	Non-inf. prog.	Inf. prog.	Non-inf. prog.	Inf. prog.	Non-inf. prog.	Inf. prog.
	Number	*Number*	*Number*	*Number*	*Per cent*	*Per cent*
Colsess IV × Trebi	1052	1305	887	955	15.69	26.82
Trebi × Colsess IV	385	772	327	612	15.07	20.72
Trebi × Missouri Early Beardless	2999	1861	2686	1619	10.44	13.01
Missouri Early Beardless × *Hordeum deficiens*	752	467	660	384	12.24	17.78
Hordeum deficiens × Missouri Early Beardless	927	594	793	468	14.46	21.22

resulting either in failure of the seed to germinate, or in the production of a weak seedling. This tends to eliminate susceptible plants in the progenies and to cause some of the segregating progenies to be classified as resistant.

TABLE 6.—*Infection of F_3 families from non-inoculated plants when inoculated with chlamydospores of Ustilago nuda*

Hybrid	Prog.	Seeds pltd.	F_3 plants	F_3 families in infection percentage classes		
				0.0	1–34.99	35–100
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Trebi × Missouri Early Beardless	172	4860	4305	104	43	25
Missouri Early Beardless × *Hordeum deficiens*	72	1212	1044	47	10	15
Hordeum deficiens × Missouri Early Beardless	96	1472	1261	61	18	17

The infection data of the hybrids from Trebi with Missouri Early Beardless, and *Hordeum deficiens* with Missouri Early Beardless are presented in table 6. The distribution of families in 5 per cent infection groups is shown in figure 2. They do not harmonize with the 1:2:1 ratio because of a preponderance of resistant progenies. The average percentage of infection in segregating and susceptible F_3 progenies of the Trebi × Missouri Early Beardless cross was also low (36.53) when compared with 52.86 per cent infection of comparable F_3 progenies from the cross, Colsess IV × Trebi. These results indicate the possible existence of a factor for resistance in the hybrids from Trebi × Missouri Early Beardless, which was not present in the Colsess IV × Trebi hybrids.

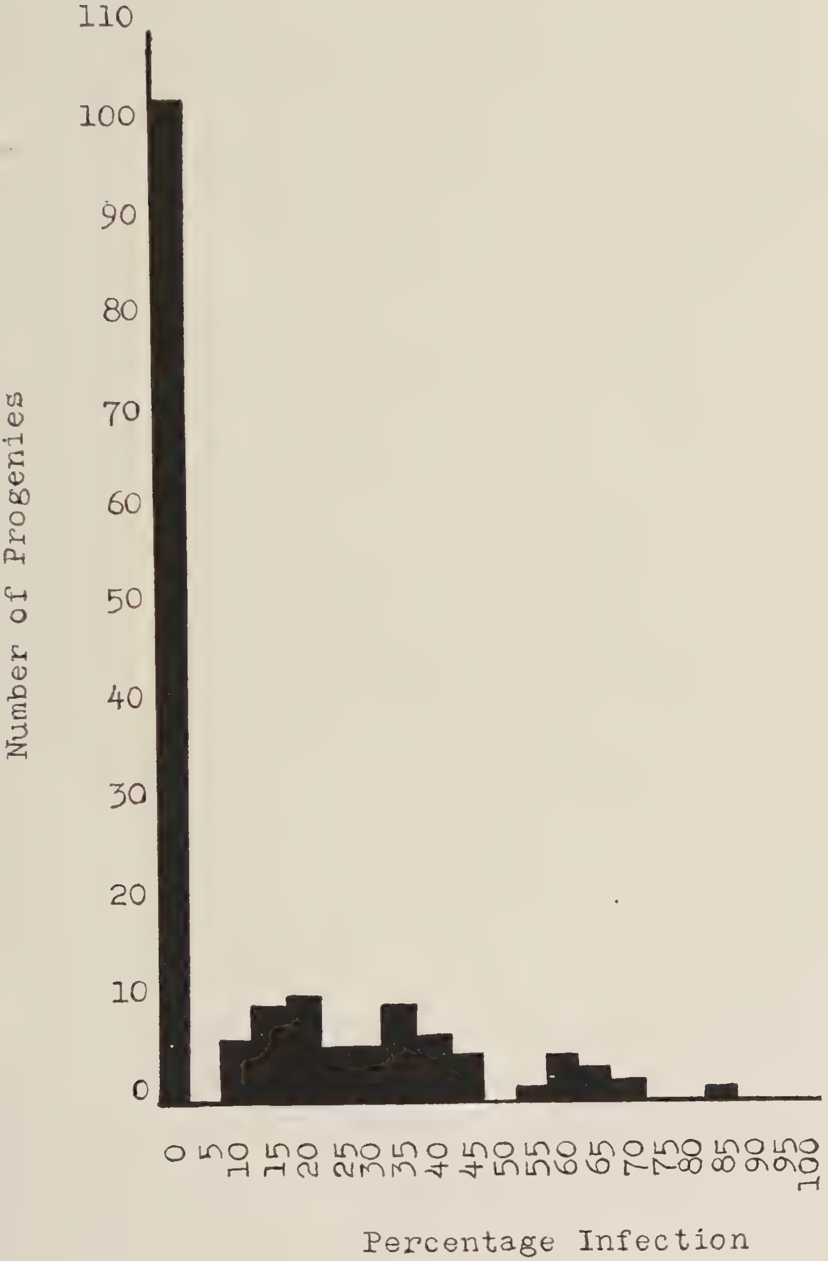

FIG. 2. Distribution of F_3 progenies in 5 per cent infection classes in the cross of Trebi × Missouri Early Beardless when inoculated with chlamydospores of *Ustilago nuda.*

The average percentages of infection of the segregating and susceptible F_3 progenies obtained when Missouri Early Beardless was crossed with *Hordeum deficiens* were nearly the same as that obtained when Missouri Early Beardless was crossed with Trebi (Table 7). The hybrids from each cross also reacted similarly with respect to the numbers of resistant, segregating, and susceptible progenies. *H. deficiens* probably possesses a single factor for resistance, which is similar in its effect to the factor carried by Trebi. The large number of resistant progenies may be due to a weak factor for resistance in Missouri Early Beardless. The existence of such a factor was indicated, also, by the behavior of Trebi × Missouri Early Beardless hybrids.

TABLE 7.—*Percentages of infection by Ustilago nuda in inoculated segregating and susceptible F_3 progenies of various barley hybrids*

Hybrid	Families	Plants	Infected plants	
	Number	*Number*	*Number*	*Per cent*
Trebi × Missouri Early Beardless	68	1619	517	31.93
Missouri Early Beardless × *Hordeum deficiens*	25	384	155	40.36
Hordeum deficiens × Missouri Early Beardless	35	468	171	36.53
Colsess IV × Trebi	62	1139	602	52.86

Backcross Progenies

The F_2 plants from the backcross were inoculated by introducing the spores of *Ustilago nuda* into the flowers of the F_1 plants. The data are presented in table 8. Of 98 inoculated F_2 families, obtained by backcrossing F_1 hybrids of Trebi × Colsess IV onto Colsess IV, 21 were free from infection, while 77 contained 1 or more infected individuals. If resistance is controlled by a single factor, all of the F_2 families from the backcross should show some infection with a tendency for the progenies to accumulate in two infection groups. If 25 per cent is arbitrarily taken as the upper limit of susceptibility of the progenies from heterozygous backcross-generation plants, 57 progenies would be classified as heterozygous and 41 as homozygous for susceptibility. These figures agree reasonably well with the expected segregation of 1 heterozygous : 1 homozygous progeny, in view of the afore-mentioned difficulties in obtaining infection of all susceptible plants.

Of the F_2 plants, obtained by backcrossing F_1 hybrids of Trebi × Missouri Early Beardless onto Missouri Early Beardless, there were 61 progenies showing less than 25 per cent infection, and only 23 progenies showing 25 per cent or more infection. This segregation does not conform to the 1 heterozygous : 1 homozygous ratio expected when a single factor for resistance is operating. The resistant parent used in this backcross was the same as that used in producing the hybrids of the backcross, Colsess IV × (Trebi × Colsess IV), which segregated reasonably close to the 1 : 1 ratio when inoculated under similar conditions. This further indicates that the Missouri Early Beardless parent probably contributes a factor for resistance that tends to reduce the susceptibility of the hybrids, in addition to the factor for resistance contributed by Trebi.

TABLE 8.—*Infection of the first-selfed generation progenies from the backcross when inoculated with chlamydospores of Ustilago nuda*

Backcross	Prog.	Plants	Progenies in infection percentage classes		
			0.0	1–24.99	25–100
	Number	*Number*	*Number*	*Number*	*Number*
Colsess IV × (Trebi × Colsess IV)	98	1766	21	36	41
Missouri Early Beardless × (Trebi × Missouri Early Beardless)	84	1219	32	29	23

ASSOCIATION OF CHARACTERS

Hybrids from the cross, Missouri Early Beardless × *Hordeum deficiens,* were used for studying the association between morphological characters. They were particularly desirable for a study of the association, since the parents differed in the expression of 3 morphological characters, important from the standpoint of development of a desirable variety of winter barley. Missouri Early Beardless possessed the 3 desirable characters, hoods, six-row heads, and winter habit, but was susceptible to *Ustilago nuda,* whereas *H. deficiens* possessed awns, *deficiens*-type heads, spring habit, and resistance to *Ustilago nuda.*

The factor pairs for the hooded *vs.* awned, and the non-six-row *vs.* six-row condition have been placed in separate linkage groups (6) and confirmed in this investigation. No reports have been found in the literature concerning linkage relations of the factors that control spring *vs.* winter habit in barley.

Segregation and linkages in the second generation were determined by observing the segregation of uninoculated F_2 progenies.

Spring *vs.* Winter Habit

F_1 Generation. All of the 36 F_1 plants of the cross, Missouri Early Beardless × *Hordeum deficiens,* showed the spring habit of growth, indicating dominance of that habit. This was true also of the first generation plants from the cross of Trebi × Missouri Early Beardless.

TABLE 9.—*F_2 segregation of spring vs. winter habit in barley hybrids from Missouri Early Beardless × Hordeum deficiens and reciprocal*

	Spring	Winter	χ^2	P
	Number	*Number*		
Obs.	161	40	2.7876	0.50
Theor. 3:1	150.75	50.25		

F_2 Generation. The progenies from 201 selfed F_1 plants were observed and the results recorded in table 9. One hundred sixty-one of the F_2 plants

were classified as spring type and 40 as winter type. When compared with the 3 : 1 ratio expected when the segregation is controlled by a single genetic factor, chi-square was 2.7876. This is a satisfactory agreement between the observed and the calculated frequencies, and indicates that a single factor pair controls the inheritance of spring and winter habit, with the factor for spring habit dominant.

Hoods *vs.* Awns with Spring *vs.* Winter Habit

F_2 segregation of hybrids from the cross, Missouri Early Beardless × *Hordeum deficiens*, indicates that the two pairs of factors for hoods *vs.* awns and spring *vs.* winter habit are inherited independently (Table 10). When compared with the normal 9 : 3 : 3 : 1 ratio, chi-square was 0.5975, indicating that the factor pairs determining the above characters are not in the same linkage group.

TABLE 10.—*F_2 association of morphological characters in Missouri Early Beardless × Hordeum deficiens hybrids*

Plant type	Spring		Winter		χ^2	P
	Obs.	Theor. 9 : 3 : 3 : 1	Obs.	Theor. 9 : 3 : 3 : 1		
	Number	*Number*	*Number*	*Number*		
		Hooded × awned				
Hooded	63	65.25	24	21.75	0.5975	> .8
Awned	23	21.75	6	7.25		
		Non-six-row × six-row				
Non-six-row	66	65.25	25	21.75	1.3332	> .7
Six-row	20	21.75	5	7.25		

Non-six-row *vs.* Six-row with Spring *vs.* Winter Habit

When the frequencies obtained in the segregation of non-six-row *vs.* six-row with spring *vs.* winter habit were compared with the normal 9 : 3 : 3 : 1 ratio expected when 2 factor pairs segregate independently (Table 10), chi-square was 1.3332. This is not a significant deviation from the values expected with independent segregation.

It may be concluded from the data presented on the segregation of morphological characters that the 3 factor pairs determining hoods *vs.* awns, non-six-row *vs.* six-row heads, and spring *vs.* winter habit are located in separate linkage groups.

Resistance *vs.* Susceptibility with Hoods *vs.* Awns

The data for the comparison of the frequencies of resistant and susceptible F_2 progenies with hooded and awned progenies are presented in table 11. Because of the difficulty of accurately classifying the resistant, segregating, and susceptible progenies, a theoretical ratio, with which linkage intensities may be compared, cannot be formulated. In an attempt, however, to obtain some indication of possible linkage, the data are compared with frequencies expected when segregation is controlled by 2 independent factor pairs.

TABLE 11.—F_2 *segregation of hoods vs. awns with resistance vs. susceptibility*

Cross	Ratio	Hoods		Awns		Total
		Resis.	Suscep.	Resis.	Suscep.	
		Number	*Number*	*Number*	*Number*	*Number*
Colsess IV × Trebi and reciprocal	Obs.	104	38	30	12	184
	Theor.	103.50	34.50	34.50	11.50	
	9 : 3 : 3 : 1					
Missouri Early Beardless × *Hordeum deficiens* and reciprocal	Obs.	110	23	26	9	168
	Theor.	94.50	31.50	31.50	10.50	
	9 : 3 : 3 : 1					
Trebi × Missouri Early Beardless	Obs.	111	22	36	3	172
	Theor.	96.75	32.25	32.25	10.75	
	9 : 3 : 3 : 1					

There was no indication of linkage between the factor pairs determining resistance *vs.* susceptibility and hoods *vs.* awns in hybrids from Colsess IV × Trebi. Segregation for resistance in hybrids from this cross was apparently controlled by a single factor, and when the data are compared with the segregation for hoods *vs.* awns, it approaches the 9 : 3 : 3 : 1 ratio expected with independent segregation of 2 factor pairs.

The data for the segregation of the factor pairs in hybrids from Missouri Early Beardless with *Hordeum deficiens* and Trebi do not agree with the 9 : 3 : 3 : 1 ratio. There were more resistant plants and fewer susceptible ones in both the hooded and awned classes than was to be expected with segregation of two independent factor pairs. The segregation of the hybrids for resistance and susceptibility indicated the presence of a single dominant factor for resistance supplied by the resistant parents and an additional weak factor for resistance furnished by the Missouri Early Beardless parent. The possible presence of this second factor and the difficulty of obtaining universal infection of the susceptible progenies probably accounts for the large number of resistant progenies in comparison to the number of susceptible ones. For these reasons it is doubtful if there is any linkage between the factor pairs determining resistance *vs.* susceptibility and hoods *vs.* awns in any of the crosses studied.

Resistance *vs.* Susceptibility with Non-six-row *vs.* Six-row

There was no indication of linkage between the factors determining resistance and susceptibility and those determining non-six-row and six-row heads. When the data are arranged in 4 classes, the numbers approach those expected on the basis of independent segregation (Table 12).

TABLE 12.—*F_2 association of characters in reciprocal crosses of Missouri Early Beardless × Hordeum deficiens*

Plant type	Resistant		Susceptible		Total
	Obs.	Theor. 9:3:3:1	Obs.	Theor. 9:3:3:1	
	Number	*Number*	*Number*	*Number*	
Non-six-row × six-row					
Non-six-row	96	95.04	21	31.68	
Six-row	40	31.68	11	10.56	168
Spring × winter					
Spring	53	47.81	20	15.93	
Winter	3	15.93	9	5.31	85

Resistance *vs.* Susceptibility with Spring *vs.* Winter Habit

The data for the segregation of the factor pairs determining resistance *vs.* susceptibility and spring *vs.* winter habit are presented in table 12.

Of the 85 inoculated F_2 plants only 3 possessed resistance and winter habit. This is a much smaller number of resistant winter plants than was to be expected, since resistance is transmitted as a dominant character. There were more susceptible winter-type than resistant winter-type individuals, suggesting the possibility of linkage between the factors for winter habit and susceptibility; however, the number of plants is not sufficiently large to permit definite conclusions.

In the field there would probably be less evidence of linkage between the factors for winter habit and susceptibility because infected embryos often produce weak plants, which would be eliminated by winter conditions. Thren (9) reported weak germination of seeds from flowers inoculated with a large number of viable spores of *Ustilago nuda;* the loss was higher in winter than in spring barley, the result of the inability of weak, infected plants to withstand adverse weather conditions.

DISCUSSION

The exact nature of the inheritance of resistance in barley to *Ustilago nuda* was not determined because of the failure to identify all susceptible and segregating genotypes. An inoculation method that will give 100 per cent infection with *U. nuda* has not yet been devised. Another complicating factor is the possibility of the presence of several physiologic forms of *U. nuda* in the inoculum, even though the inoculum supply were built up from a single infected head of Missouri Early Beardless barley by repeated inoculation of that variety. A third factor, previously pointed out by Roemer (7) and Thren (9), is that infected plants may be weakened by the presence of the loose-smut fungus, which results in a selective death of seedlings. This difficulty was partly eliminated in the present investigation by germinating the seeds in the laboratory, planting in plant bands, and growing to a height of 3 inches in a cold frame before transplanting to the field. Progenies that survived the winter in the field were, however, undoubtedly subject to loss of infected seedlings, possibly accounting, in part, for the preponderance of resistant plants and progenies.

It was apparent, however, that resistance to *Ustilago nuda* was inherited as a dominant character. The F_1 generation usually equalled or approached the resistance of the resistant parents, although in crosses between Trebi and Colsess IV there was some evidence that dominance may not be complete. In later generations there was apparently an excess of resistant plants. Nahmmacher (5) and Zeiner (10) found resistance to *U. nuda* was completely dominant and controlled by a single factor in certain barley crosses.

Resistance appeared to be physiological. When flowers were inoculated on a plant carrying factors for resistance in a heterozygous condition, both resistant and susceptible plants were obtained. Hyphae from the fungus spores must reach the ovule in order to infect the susceptible embryos. Except for the tissues that developed following fertilization of the egg nucleus within the embryo sac, the tissues of the flowers that produced resistant and susceptible plants were the same. Therefore, the hyphae must be able to penetrate the floral tissues to the embryo in plants carrying factors for resistance in the dominant condition. The infection of the F_1 plants of reciprocal crosses (Table 2) also suggests that the fungus reaches the embryos with approximately equal frequency, whether the female parent is resistant or susceptible. Larose and Vanderwalle (2) and Milan (3) found this true of the infection of F_1 progenies of reciprocal crosses between very susceptible and very resistant wheat varieties inoculated with *Ustilago tritici*. The resistance of the F_1 plants approached that of the resistant parent, regardless of the resistance of the female parent.

SUMMARY

The inheritance of resistance to the brown loose smut of barley, caused by *Ustilago nuda*, was studied in barley hybrids. The two susceptible varieties, Missouri Early Beardless and Colsess IV, and the resistant varieties Trebi and *Hordeum deficiens* were used as parents.

Infection reactions of F_1 plants from reciprocal crosses of Trebi with Colsess IV indicated that the infection tubes from the chlamydospores of *Ustilago nuda* reached the hybrid embryos with approximately equal frequency, regardless of the resistance or susceptibility of the female parent.

Both Trebi and *Hordeum deficiens* apparently possess a dominant factor for resistance; but dominance may not always be complete. F_2 progenies from crosses of each variety with Missouri Early Beardless were infected to a similar degree, indicating that the resistance factors carried by the 2 resistant parents were similar in their effects.

In F_2 and subsequent generations there was a preponderance of resistant plants, possibly attributable to lethal effects of infection, to variations in percentages of infection obtainable by the methods used, and to failure to obtain universal infection of the susceptible parent.

The susceptibility of F_2 hybrids from a Colsess IV × Missouri Early Beardless cross approached the infection limits of Missouri Early Beardless but was less than that of Colsess IV, suggesting the occurrence of a weak resistance factor in Missouri Early Beardless.

The susceptibility of first-selfed backcross generation progenies was in agreement with the susceptibility of F_2 and subsequent generations, offering additional evidence for the presence of a single dominant factor for resistance in Trebi and *Hordeum deficiens*, and possibly a weak factor for resistance in Missouri Early Beardless.

There was no evidence of linkage between the factors for resistance and those for hoods or six-rowness.

Promising selections of hooded, six-row, winter barleys resistant to *Ustilago nuda* were obtained.

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A METHOD FOR THE CULTURE OF SEEDLINGS AND SMALL PLANTS IN SUNLIGHT UNDER CONTROLLED TEMPERATURE CONDITIONS[1]

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(Accepted for publication August 4, 1941)

INTRODUCTION

The effect of ever-changing environment upon growing plants is a perennial problem confronting nearly all botanical investigators. To circumvent the problem numerous techniques have been adopted for stabilizing one or more environmental factors, or to avoid them completely, depending upon the nature of the problem, the facilities available, and the degree of accuracy required. Apparatus of many sorts have been described, but most of them represent an accumulation of expensive equipment requiring shop tools and the services of specialized mechanics and skilled craftsmen for construction and maintenance. Many small laboratories with limited funds and personnel, cannot afford the expenditure of more than a few hundred dollars over a period of years for temperature-control equipment.

The purpose of this paper is to describe a refrigerated plant-culture chamber designed to meet the needs of small-scale greenhouse research. By taking advantage of local commercial refrigeration sales and service the cost may be held to a minimum without sacrificing accuracy and efficiency.

PRINCIPLES OF DESIGN

Since sunlight, passing through glass, seemed to be the most practical means of illumination for growing plants in controlled environments, the chambers were designed for installation in a section of greenhouse with thermostatically controlled heat (Fig. 1). On a bright summer day, when greenhouse temperatures approximate 100° F., without roof shade, the inside cabinet temperature of an unrefrigerated empty chamber may reach 150° F. If a cloud passes between the sun and the chamber, the temperature in a well-ventilated greenhouse may fall several degrees but that of the unrefrigerated chamber may remain exceedingly high depending upon the efficiency of its insulation. Heat of radiation resulting from the sun's rays remains trapped within the insulated chamber, since little of it is conducted through well-insulated walls. This accumulated heat is of little importance, since it arises from surfaces heated by light rays. If surfaces within the chamber are reduced to a minimum and if these are leaf surfaces, as they should be, for optimum efficiency, the rate of radiation, convection, and accumulation of heat in a small plant-growing chamber is not too great to be dissipated by ordinary refrigeration equipment.

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[1] Published with the approval of the Director of the Virginia Agricultural Experiment Station.

[2] Formerly Associate Plant Pathologist and Senior Agricultural Scientific Aide, respectively, Virginia Agricultural Experiment Station. The writers also wish to express their gratitude to F. A. Wolf, W. A. Whitney and others for assisting in many ways.

If air be passed across these leaf surfaces at low velocity, convected heat of radiation is easily swept away where it may be cooled. Thus, if the design of a plant-growing chamber be such that a maximum working surface of leaf area is exposed to sunlight, together with a minimum surface of heat-absorbing structural materials, low-velocity air circulation may be employed with a minimum of refrigeration equipment. By reducing the non-working heat-absorbing surfaces (exposed structural materials) in a chamber one may obtain also a better balance between chamber-air temperature and cooling-coil temperature, which facilitates more accurate control.

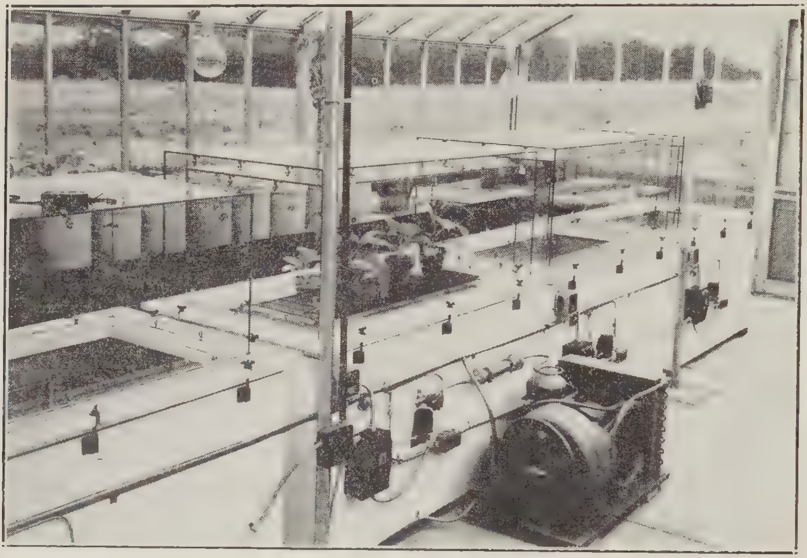

FIG. 1. A battery of 4 plant-culture chambers installed in the experimental greenhouse. Artificial illumination supplemented sunlight when necessary. The compressor assembly, dehydrator, shut-off valve, and solenoid stop valve, all in the small "liquid" line, are shown in the foreground. (See Fig. 2.)

The basic design for the chambers described in this paper, therefore, consisted of exposing plants to sunlight under glass and sweeping radiated heat away by low velocity air movement. The apparatus used and the results obtained are described below.

MATERIALS AND METHODS

Each insulated cabinet (I. C., Fig. 2) was constructed in two parts, the lower of wood, with cork insulation between the walls, and the upper of glass plates separated by an air space. The lower part of each cabinet (Figs. 2, 3) was provided with a refrigerator door closing against two rubber gaskets. The interior of each box was lined with galvanized iron, soldered at the seams. A small drain pipe, soldered to the lining and extending

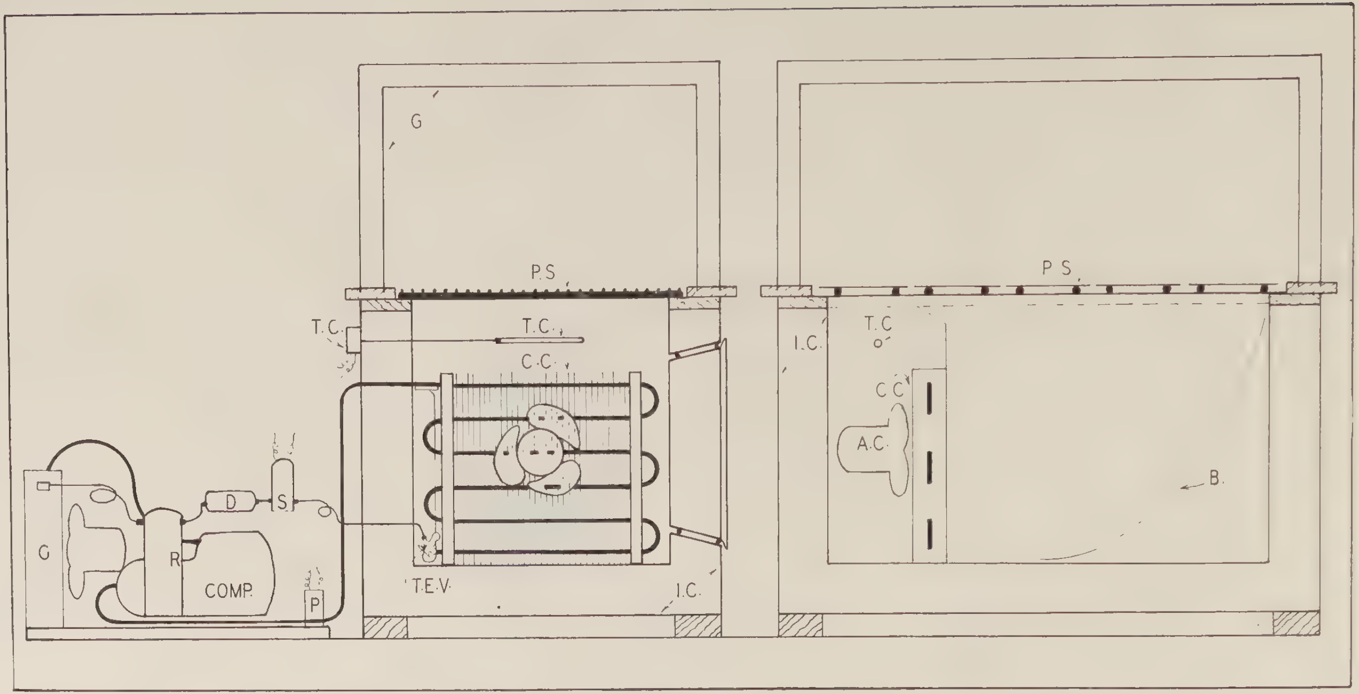

FIG. 2. Diagram of apparatus employed for the culture of seedlings and small plants in sunlight under controlled temperature conditions. A. C., air circulator; B, baffle; C, condenser; C. C., cooling coil; Comp., compressor; D, dehydrator in "liquid" line (positive pressure); G, glass; I. C., insulated cabinet; P, pressure switch in "suction" line (negative pressure); P. S., plant supports; T. C., temperature controller; T. E. V., thermostatic expansion valve.

through the bottom, facilitated cleaning. It also relieved interior gas pressures caused by temperature changes or by opening or closing the door. Several hinged wing bolts, attached to the top of the box (Fig. 3), permitted secure fastening of the glass section. A rubber gasket between the box and the glass section insured a leak-proof union.

The glass portion of the chambers was seated in rubber-filled channels in a wood frame. The plates of glass were cemented at the edges with waterproof show-case cement of suitable quality. Show-case clamps were used to hold the glass in place. The above method was adopted to minimize shading, which other more bulky structural materials would cause if used to hold the glass in place. Suitably placed holes in the wood frame supporting the glass permitted escape of air entrapped between the two glass walls. Tubes of a suitable drying agent connected to these small openings prevented moisture from collecting on the glass between the walls in case a leak in the glass union should develop.

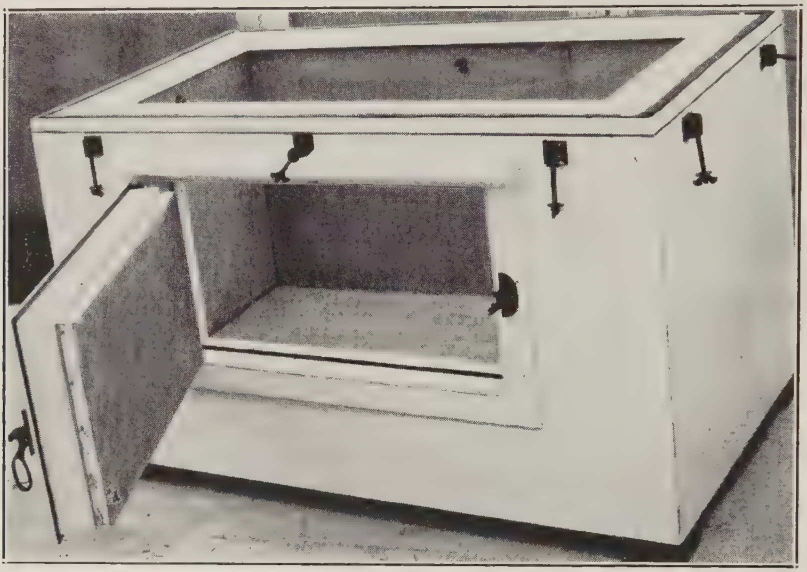

FIG. 3. Lower portion of insulated cabinet (I. C.) consisting of a metal-lined, cork-insulated, wooden box with refrigerator type door.

The outside dimensions of the cabinets herein described were approximately 48 in. long by 33 in. wide by 51 in. high. An unobstructed surface approximately 38 by 36 in. was available for plants growing in flats. By rearranging the plant supports (P. S., Fig. 2), potted plants up to 36 in. high could be introduced, though with some inconvenience.

Iron grids, made in small sections for easy removal, were used to support the flats of soil or potted plants. The plants were introduced through the door of the chamber and raised into position on the grid. Space at each end of the chamber was kept clear to allow free circulation of air against the chamber walls. The success of this type of chamber depends upon the movement of large volumes of air at low velocity across all interior surfaces.

The unassembled apparatus, used for controlling temperatures within each chamber, is shown in figure 4. A diagram of the assembled apparatus, connected to the compressor unit, is shown in figure 2. A description of each unit of the apparatus follows:

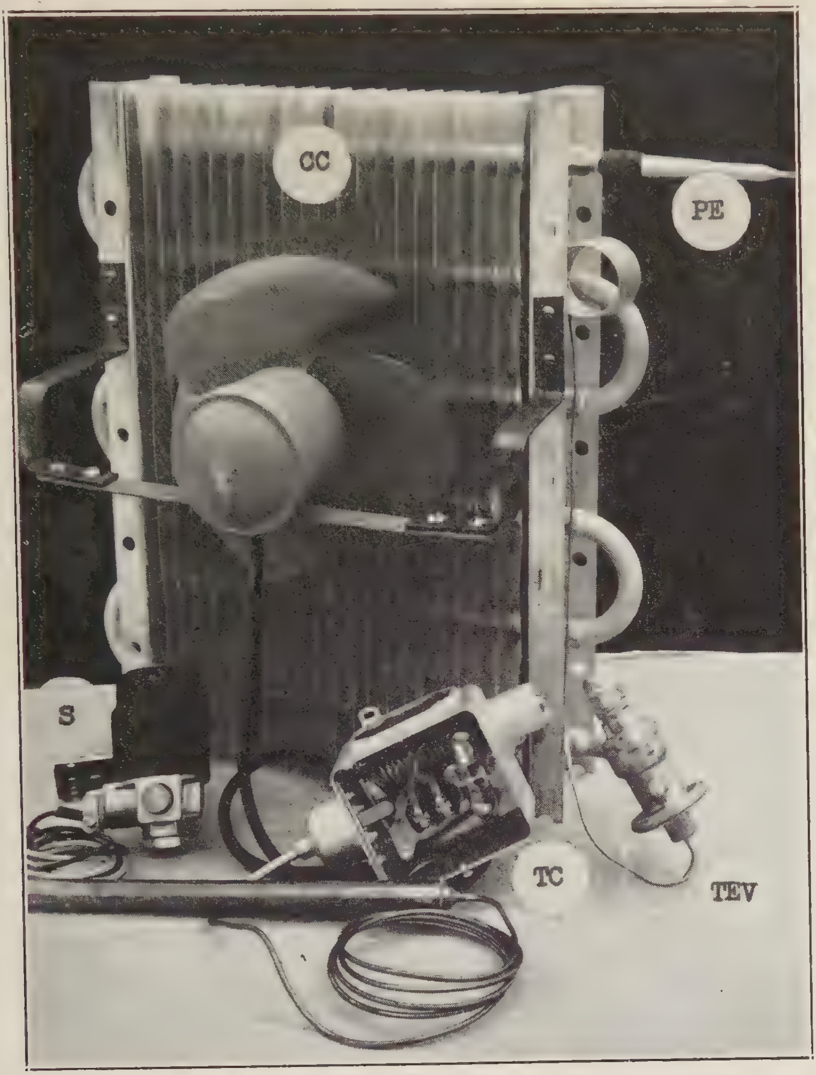

FIG. 4. Refrigeration apparatus required for one plant chamber. C. C., cooling coil; T. E. V. thermostatic expansion valve with its power element; T. C., temperature controller; S, solenoid stop valve.

The condensing unit,[3] or compressor assembly (cf. Fig. 2), used to operate all 4 chambers consisted of a compressor and a one-half horsepower motor hermetically sealed. The refrigerant employed was ''Freon-12'' (difluorodichloromethane, CF_2Cl_2). If the average refrigerant temperature used is 40° F. and if the surrounding air temperature is 100° F., the above unit, if operated continuously, was rated at 7120 BTU an hour. This rating is equivalent to 1186 pounds of ice a day.

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[3] Model AFE–50, manufactured by Westinghouse Electric Company.

Upon condensing the gaseous refrigerant in the condenser (C., Fig. 2) it was collected in receiver R, and held under pressure by means of suitable automatic values at S, and one within the compressor itself. A vacuum was thus established at valve P.

A rise in temperature within the chamber caused the temperature controller bulb T. C. to close an electrical circuit that opened the solenoid stop valve S. Liquid refrigerant then passed from receiver R, through dehydrator D, into the thermostatic expansion valve T. E. V. This valve, operated by means of its power element, attached to the upper tube of the cooling coil, permitted liquid refrigerant to enter the bottom of cooling coil C. C. until the lowest temperature of the cooling coil reached a predetermined value. For ordinary work this valve was adjusted to close at a temperature above freezing, thus preventing frost from collecting on the coil and subsequent dehydration of the chamber air. By carefully adjusting the thermostatic expansion valve T. E. V., when the chamber was in normal operation, sudden temperature changes were avoided, since the temperature of the coil could be regulated to slightly below that required for the chamber. Under ideal conditions this adjustment should operate the cooling coil at a temperature sufficiently below that of the chamber to compensate for heat loss through the chamber walls. If very low temperatures are required, the temperature difference between the coil and the chamber must be increased. The thermal stability of a well-insulated chamber, the door of which is opened infrequently, is scarcely affected by external room temperatures when the coil temperature is balanced with the chamber heat loss.

As refrigerant enters the lower part of the cooling coil it expands into a gas and cools the coil. As pressure is increased in the cooling coil, valve P is caused to close an electrical circuit which starts the compressor and reestablishes positive pressure at point S and negative pressure at P.

A slow-speed air circulator A. C., operated by an induction motor, caused low-velocity air movement across the various surfaces within the chamber.

Humidifying and dehumidifying equipment of various sorts was used by placing the apparatus in the air stream on the bottom of the chamber between the cooling coil and the rubber curtain baffle B.

With the greenhouse temperature thermostat adjusted at 75° F., refrigeration was required in all chambers operating below this level, while heat was usually required above this level at night and on cool, cloudy days. During periods of bright sunshine, and regardless of the greenhouse temperature, refrigeration was usually required in all chambers. By means of an auxiliary heater one chamber was operated very successfully at 105° F.

Refrigeration was required in this chamber during part of the mid-day if bright sunshine prevailed.

The cost of a single cabinet with all equipment was approximately $200. The cost of the compressor assembly complete, was approximately $180. Power consumption during continuous operation was approximately 900 watts an hour.

RESULTS AND DISCUSSION

Although the chambers described in this paper were exposed to sunlight as much as possible in a warm greenhouse, temperatures of the air and soil were held to within approximately 2° or 3° F. of a fixed value. The range of temperature over which these chambers were tested in full sunlight lay between 65° and 105° F. Somewhat higher or lower temperatures could undoubtedly have been reached. Figure 5, A and B, shows the thermograph record of two chambers operating under the above-described conditions. A third chamber, C, was adjusted to operate for a 14-hour period during the night at a temperature of between 25° and 27° F. In this chamber no attempt was made to regulate the upper (daytime) temperature except to prevent it from rising above 70° F. All three chambers were in operation continuously over a period of many weeks. No attempt was made to regulate the greenhouse temperatures in which the chambers were located except to maintain a minimum of 75° F. and to provide ventilation on warm days.

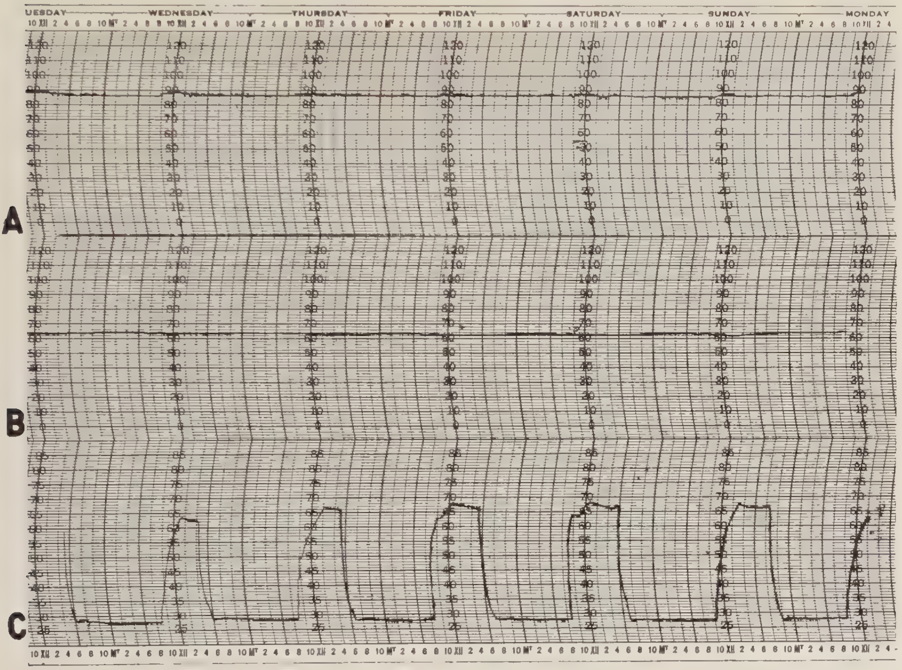

FIG. 5. Thermograph records of the sub-surface soil temperatures. A, 85° to 87° F. chamber; B, 65° to 67° F. chamber; C, 27° to 29° F. chamber. The latter was adjusted nightly for a 14- to 15-hour low-temperature treatment.

Air temperatures, within the chambers, varied, depending upon the opening and closing of the temperature-controller switch. During periods of bright sunshine and intense radiation, the air temperature at a fully illuminated soil surface was frequently as much as 2 or 3 degrees below the subsurface soil temperature. During periods of cloudy weather or when none of the sun's rays reached the soil, air temperatures were slightly above those of the sub-surface soil layers. Excluding the rate of heat conduction through dry soils, it appears probable that heat exchange, between the soil surface and the air stream immediately above, is affected significantly by surface soil moisture and the amount of moisture in the air blanket immediately above. The use of an appropriate soil cover, such as ground cork, may prove useful in obtaining closer agreement between soil and air temperatures in this of type chamber.

Growth of tobacco seedlings under three thicknesses of glass (two in the chambers and one in the greenhouse) did not seem to be as good as check plants grown on the greenhouse bench. While allowances were made for temperature variation, the quality of the foliage was noticeably different. By providing a longer day for the plants in the chambers, these differences were no longer apparent. The response of Burley and Turkish tobacco seedlings to the temperatures shown in figure 5, A and B, for a period of two weeks[4] is shown in figure 6. The soils in which these seedlings were growing was infested with the black root-rot fungus, *Thielaviopsis basicola* (Berk.) Ferraris. One "acid" soil (pH 5.1) and one "neutral" soil (pH 7.3) was used for each tobacco variety grown at each temperature. This illustration shows the interesting relation between varietal susceptibility, and soil acidity, in a controlled environment. The Burley variety, Judy's Pride, grew very poorly in the acid soil at 65° F., while the Turkish variety, Xanthi, made somewhat better growth. Similar relative growth was made by the two varieties at 85° F., although the Burley variety was more sensitive to the acid soil than was the Turkish variety. In neutral soil at 65° F., the Burley variety was attacked by the root-rot fungus and plant growth was reduced. The Turkish variety, being nearly immune from black root rot, made normal growth. At 85° F., the black root-rot pathogen was not a retarding factor of growth for either variety, although the retarding effect of soil acidity was quite apparent.

During the construction and operation of these chambers the following improvements were suggested that seem worthy of recording: 1. The chambers might be located out of doors to avoid one thickness of glass, shading by roof rafters and supports, radiated heat from cement floors, soil benches, and the like. 2. The use of clear plastic materials as a substitute for glass would permit lighter construction, easier access to plants, and the use of the entire sun's spectrum for illumination. 3. Built-in heating, humidifying, and plant watering devices. 4. Employment of a heat exchanger in each chamber of the type described would improve refrigeration efficiency.

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[4] Twenty-four seedlings, the largest leaves of which were one-half inch in diameter were spaced two inches apart in each flat.

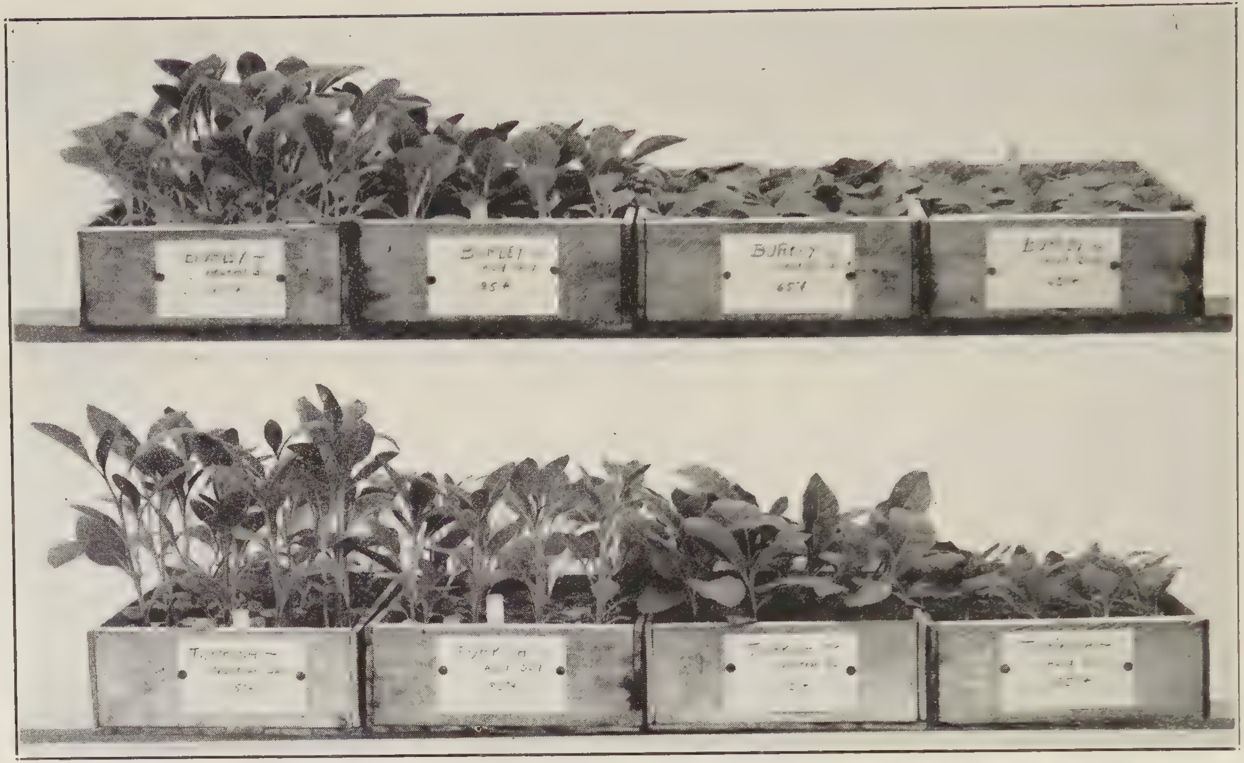

FIG. 6. Response of 24 Burley (upper) and 24 Turkish (lower) tobacco seedlings after a 2-weeks exposure to temperatures approximating 85° and 65° F. N, "neutral" soil; A, "acid" soil, both infested with tobacco black root rot fungus (*Thielaviopsis basicola*). The largest leaves of each plant at the beginning of this experiment were one-half inch. Distance between plants was 2 inches.

SUMMARY

Refrigerated plant-culture chambers are described for use in full sunlight between temperatures of 65° and 105° F., and in darkness at temperatures as low as 25° F.

Ordinary commercial refrigeration equipment, apparatus, and materials were employed for simplicity and economy.

The accuracy of temperature control obtainable with the commercial equipment used was within 2° or 3° F. of that desired.

The principle of design, adopted for these chambers, may be described as the movement of low-velocity air at a stated temperature across surfaces exposed to light radiation.

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CONTROL OF SEEDLING DISEASES OF SUGAR BEETS IN MONTANA[1]

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(Accepted for publication September 4, 1941)

Seedling diseases have considerable importance in the growing of sugar beets, especially on the heavy irrigated soils of Montana. Young beet seedlings are usually attacked by different pathogenic organisms either at the time when they emerge from the seed balls or later when they have emerged above ground.

Three more or less distinct types of seedling diseases of sugar beets have been described by Coons and Stewart (3). Seedlings with the first type of disease show a browning and blackening of the hypocotyl and root. The discoloration usually shows above the ground and the killing of the seedlings varies from fairly rapid to very slow. A plant may frequently show its hypocotyl completely blackened. The cotyledons, however, remain turgid and green and, on examination, the vascular region of the plant appears to be the only part not affected. *Phoma betae* is chiefly associated with this type of disease and is usually seed-borne.

Seedlings affected by the second type of disease wilt promptly, but usually show no marked discoloration. Such plants are characterized by a brown decayed region in the root, and the central vascular region is discolored far in advance of the external lesions. The lesions have a water-soaked appearance in contrast to the dry, black areas typical of the first type of disease. Twenty-four hours after the first indication of wilting appears the seedling is almost completely decayed. *Pythium* spp. and also other soil-borne phycomycetous fungi are associated with this type of disease.

In the third type of beet seedling disease, the leaves usually show a green or blue-green and the stem a lemon-yellow color. The seedlings grow slowly and, in general, show evidence of malnutrition. The taproot usually is found decayed at the tip, and rootlets appear, apparently, to replace the primary root. *Rhizoctonia* spp., which are also soil-borne, are chiefly responsible for this type of disease.

Most of the beet seedling diseases occurring in Montana resemble more closely the first type, while the second and third types occur only to a limited extent. Up to the present, however, it has not been proved that only *Phoma* is responsible for seedling diseases in this State.

Seedling diseases of sugar beets, at least from the standpoint of the causal organisms, usually are of a complex nature and often several organisms may be responsible for the disease. A slight difference in the composition of the soil, its temperature and other factors may be responsible for a variation in the pathogens responsible for diseases of crops (7).

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[1] Contribution from Montana State College, Agricultural Experiment Station. Paper No. 151 Journal Series.

[2] The authors are indebted to Mr. Dan Hansen, Superintendent of the Huntley Branch Station and to Mr. Stephen H. Hastings, Principal Agronomist, Bureau of Plant Industry, U. S. D. A., for their interest in this study and for furnishing assistance and facilities during this investigation.

The seedling-disease problem is complicated by the fact that it is rather difficult to isolate from seedlings of the sugar beet the organism mainly responsible for the diseases. Usually there are present, with the primary organisms, secondary ones that grow much faster on ordinary media used for isolations than do the pathogens responsible for the disease. Coons and Stewart (3) found the same difficulties in obtaining isolations of the pathogens responsible for seedling diseases. Numerous isolations from diseased seedlings in Montana were made and the results of the detailed studies on them will be reported in a subsequent paper.

Previous investigations of seedling diseases of sugar beets conducted in the rotation plots at the Huntley Branch Station, in Montana, indicated that the occurrence of these diseases is closely associated with the productive power and physical conditions of the soil, and with the other crops grown in the rotations. Weather and many other factors also may have their effect on the diseases.

SOIL AND SEED SANITATION

Soil and seed-treatment experiments were conducted in 1939 and 1940 at the Huntley station to secure additional data on the relationship between occurrence of seedling diseases of sugar beets and their environment and, also, to develop measures for the control of these diseases.

The land selected for the experiments had been alternately cropped for a number of years with barley and sugar beets, and was so depleted of its fertility that, in 1938, only 6.76 tons of beets per acre were produced.

Seed Treatments

To study the effect of disinfectants on the control of seedling diseases, one 20-lb. lot of sugar beet seed was treated with 4 oz. of Ceresan and another 20-lb. lot with 1 oz. of New Improved Ceresan by thoroughly mixing the disinfectants and seeds in a closed container.

Soil Treatments

The soil amendments were nitrates (N), phosphates (P), manure (M), and lime ($Ca(OH)_2$), applied in the following combinations:

1. NPM; 2. $\frac{N}{2}$PM. In this treatment half of the nitrates were applied at the time of planting and the other half as a side dressing immediately after thinning. 3. NP; 4. $\frac{N}{2}$P. Nitrates were applied in the same way as in 2. 5. $\frac{N}{3}$P. In this treatment, one-third of the nitrates were applied at the time of planting, one-third as a side dressing immediately after thinning, and the remaining one-third about a month later. 6. MP; 7. N; 8.

P; 9. M; 10. $Ca(OH)_2$; 11. NPM + $Ca(OH)_2$; 12. NP + $Ca(OH)_2$; 13. N + $Ca(OH)_2$; 14. P + $Ca(OH)_2$; 15. M + $Ca(OH)_2$; 16. Check.

In both years the nitrogen used was in the form of $Ca(NO_3)_2$, which was applied at the rate of 250 lb. per acre, and contained 38.75 lb. of N, while the phosphorus was a treble superphosphate for which the application rate was 175 lb. per acre, containing 80.50[3] pounds of P_2O_5. Lime in the form of $Ca(OH)_2$ was used and applied at the rate of 1000 lb. per acre. In 1939 manure was applied on the basis of 14.75 tons per acre, and, in 1940, on the basis of 22 tons per acre.

Soil treatments were used in 3 randomized replications, so that, altogether, there were 48 individual plots, each consisting of 3 randomized rows

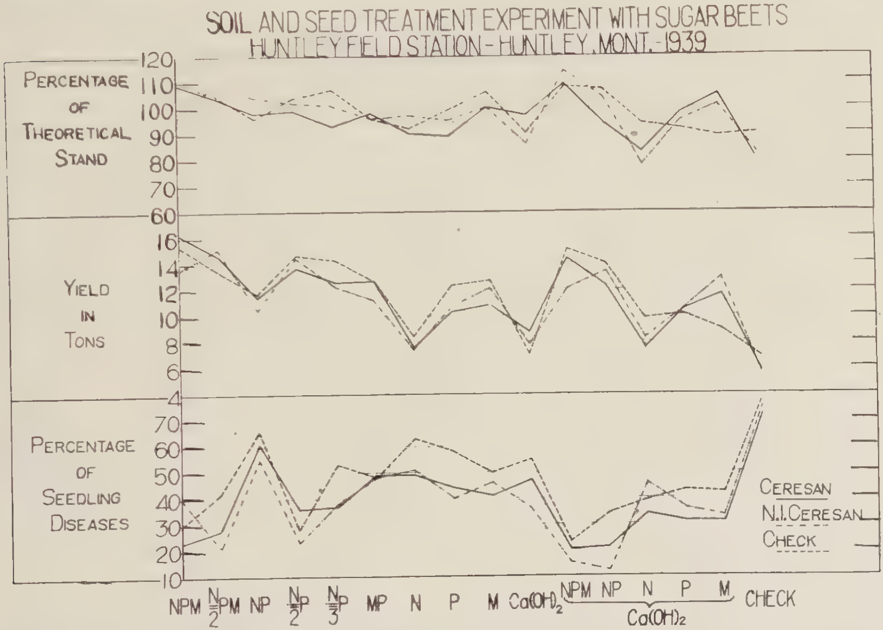

FIG. 1. The percentage of theoretical stand, ton yield, and percentage of seedling diseases of sugar beets obtained from soil and seed-treatment experiments conducted in 1939 at Huntley (Montana) Field Station.

of beets, 125 feet long. One row was planted with seeds treated with Ceresan, another with seeds treated with New Improved Ceresan, and the third with untreated seed. One buffer row was planted between each 3-row plot.

Preparation of Land

The land was plowed in the fall of 1938, double-disked and harrowed in the spring of 1939, after which lime and manure were broadcast and the soil was cultivated. The rows where sugar beets were to be planted were marked, and treble superphosphate and calcium nitrate were applied by hand in strips 3 to 4 inches wide in the center of these rows; these strips were lightly cultivated and the rows seeded. The same procedure was followed for the 1940 crop as in 1939, except that manure was applied in the fall of 1939 just before plowing. A Planet Junior hand planter was used for seeding in 1939 and a regular beet planter in 1940. The early part of the spring of 1939 was so dry that it was necessary to irrigate all beets before emergence. Later, however, there was abundant rain (7.5 inches) between May 20 and June 20. In 1940 there was sufficient spring rainfall to make early irrigation unnecessary.

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[3] According to the guarantee by the manufacturer.

Disease Readings

Sugar-beet seedlings in large numbers were examined for disease in 1939 and 1940 when the plants were in about the six-leaf stage, when beet-seedling diseases usually are at their maximum intensity. Preemergence diseases usually were responsible for only a slight loss. At harvest each row was dug, its tops and roots were weighed separately, and disease readings recorded. The results of the disease readings and the yield and stand for each individual soil and seed treatment for 1939 and 1940, respectively, are shown in figures 1 and 2 and tables 2 and 3. The data represent the averages of 3 replications.

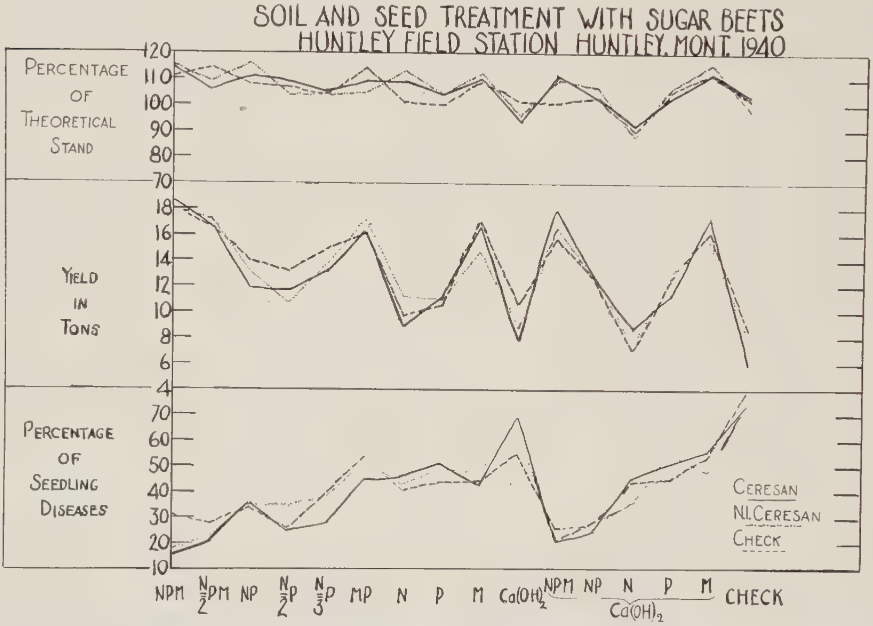

FIG. 2. The percentage of theoretical stand, ton yield, and percentage of seedling diseases of sugar beets obtained from soil and seed-treatment experiments conducted in 1940 at Huntley (Montana) Field Station.

EXPERIMENTAL RESULTS

Seed Treatments

The seed treatments (Table 1) had but slight effect on the seedling diseases, on the yield or on the stand of the sugar beets as compared to the check.

TABLE 1.—*The average results from the different seed treatments on all kinds of soil treatments in 1939 and 1940*

Seed treatments	Seedling diseases		Yield		Stand	
	1939	1940	1939	1940	1939	1940
	Per cent	*Per cent*	*Tons*	*Tons*	*Per cent*	*Per cent*
Ceresan	38.9	40.9	11.30	12.92	96.6	105.9
N. I. Ceresan	38.4	41.6	11.10	13.03	97.5	106.4
Check	46.8	41.8	11.70	13.38	99.1	105.2

The Effect of Soil Amendments, Singly and in Combinations, on the Amount of Seedling Diseases, Yield, and Stand of Sugar Beets

Sugar beets planted in plots treated either with nitrates or phosphates, separately or in combinations with $Ca(OH)_2$, had a considerable amount (av. 40.5 to 48.6 per cent) of seedling diseases, fair yields (av. 8.2 to 11.3 tons) the lowest being in the plots treated with nitrates alone (av. 8.9 tons) and in combination with $Ca(OH)_2$ (av. 8.2 tons), and, in general, they produced relatively poor stands.

Four different plots were treated with equal amounts of nitrates and phosphates, the only difference being in the method of applying the former, while one of the plots also had an application of $Ca(OH)_2$. The amount of seedling disease in these 4 plots in 1939 and 1940 was highest (60.7 and 34.9 per cent) in the plots where all nitrates were applied at seeding time while the lowest (22.2 and 26.8 per cent) followed use of nitrates in combination with $Ca(OH)_2$. In both years, less disease was recorded in plots in which nitrates were divided into 2 applications (29.2 and 28.6 per cent) than in those with 3 applications. In the latter the percentage of seedling disease (42.8 and 34.9 per cent) closely approached that of the plots in which all the nitrogen was applied at seeding time. All of these plots showed less disease than did those to which nitrates and phosphates were applied separately. There was little variation in yield and stand in these plots in both years, but they were slightly higher than those where either nitrogen or phosphates were used alone.

Sugar beets grown in the plots treated with manure, manure and $Ca(OH)_2$, and manure in combination with phosphates showed a considerable amount (average 45.5 to 49.1 per cent) of seedling diseases in both years. The yields and stands in 1940 were considerably higher than those in 1939.

Sugar beets in plots treated with either a combination of nitrates, phosphates and manure, or this combination with $Ca(OH)_2$; or with nitrates, phosphates and manure, where the nitrates were divided into two applications, manifested the minimum of seedling disease (av. 21.0 to 27.1 per cent), the highest yields (av. 15.3 to 16.7 tons per acre) and a very good stand of beets of all treatments in both years. The yields of nearly all these plots in 1940 were considerably higher and the amount of disease considerably

TABLE 2.—*Experiments with sugar beets showing the amounts of seedling diseases, theoretical stand, and yield per acre*[a] *1939*

Soil treated with	Percentage seedling diseases from seeds treated with				Yield in tons per acre from seeds treated with				Percentage theoretical stand[b] from seeds treated with			
	Ceresan	New Impr. Ceresan	Check	Average	Ceresan	New Impr. Ceresan	Check	Average	Ceresan	New Impr. Ceresan	Check	Average
NPM	22.8	39.6	29.9	30.7	16.27	13.57	15.39	15.1	109.3	95.7	110.1	105.0
$\frac{N}{2}$ PM	27.6	20.7	41.9	30.1	14.74	15.23	13.49	14.5	104.3	108.5	105.1	106 0
NP	61.4	54.9	65.9	60.7	11.45	10.53	11.69	11.2	97.9	103.5	96.3	99.2
$\frac{N}{2}$ P	36.3	23.0	28.3	29.2	13.66	14.47	14.67	14.3	98.9	102.4	104.3	101.9
N [illegible] P	37.4	38.2	52.8	42.8	12.58	12.25	14.27	13.0	93.1	101.3	106.9	100.4
MP	48.9	47.9	47.5	48.1	12.65	11.20	12.67	12.2	98.1	95.8	95.7	96.5
N	48.5	51.2	62.5	54.1	7.56	7.43	8.35	7.8	89.9	96.8	92.3	93.0
P	43.7	39.5	57.6	46.9	10.30	10.70	12.32	11.1	88.8	95.2	98.7	94.2
M	41.1	46.2	50.3	45.9	10.77	12.10	12.69	11.9	100.0	100.3	105.8	102.0
$Ca(OH)_2$	47.3	36.1	54.9	46.1	8.69	7.81	7.04	7.8	96.5	85.6	89.9	90.6
NPM + $Ca(OH)_2$	19.5	15.1	23.1	19.2	14.43	12.13	15.07	13.9	109.1	114.1	108.3	110.5
NP + $Ca(OH)_2$	20.9	11.9	33.8	22.2	12.25	13.43	13.99	13.2	93.9	104.3	106.9	101.7
N + $Ca(OH)_2$	33.5	46.3	38.9	39.6	7.48	8.31	9.78	8.5	83.3	78.1	94.1	85.2
P + $Ca(OH)_2$	30.7	36.4	42.7	36.6	10.37	10.54	10.09	10.3	98.1	94.7	92.0	94.9
M + $Ca(OH)_2$	30.9	32.9	41.5	35.1	11.62	12.86	8.91	11.1	105.1	101.3	88.8	98.4
Check	72.4	75.1	77.7	75.1	5.72	5.64	6.80	6.1	78.9	82.4	89.6	83 6

[a] All figures represent averages of three replications.

[b] Based on stand of 26,136 per acre.

TABLE 3.—*Experiments with sugar beets showing the amounts of seedling diseases, theoretical stand, and yield per acre*[a] *1940*

Soil treated with	Percentage seedling diseases from seeds treated with				Yield in tons per acre from seeds treated with				Percentage theoretical stand[b] from seeds treated with			
	Ceresan	New Impr. Ceresan	Check	Average	Ceresan	New Impr. Ceresan	Check	Average	Ceresan	New Impr. Ceresan	Check	Average
NPM	16.2	18.1	31.0	21.8	18.57	18.00	18.12	18.23	114.1	115.2	110.9	113.4
$\frac{N}{2}$ PM	21.2	22.6	28.2	24.0	16.67	17.18	16.65	16.83	105.9	108.5	113.9	109.4
NP	36.1	34.9	33.6	34.9	11.91	13.14	13.98	13.01	111.2	116.0	107.7	111.6
$\frac{N}{2}$ P	25.4	34.5	26.0	28.6	11.68	10.72	13.16	11.85	108.8	103.5	106.9	106.4
$\frac{N}{3}$ P	27.8	37.8	39.2	34.9	13.23	13.67	14.93	13.94	104.8	103.5	104.3	104.2
MP	44.5	51.7	53.7	50.0	16.33	17.16	16.08	16.52	109.3	104.5	113.9	109.2
N	45.7	43.1	40.6	43.1	8.86	11.19	9.70	9.92	108.5	113.3	101.3	107.7
P	51.3	49.2	43.8	48.1	11.22	11.02	10.51	10.92	104.3	103.7	99.7	102.6
M	43.1	48.1	43.9	45.0	16.69	14.65	17.00	16.11	110.1	112.0	108.5	110.2
$Ca(OH)_2$	69.4	65.1	55.2	63.2	7.78	8.74	10.61	9.04	93.9	96.3	101.3	97.2
NPM + $Ca(OH)_2$	21.4	21.1	25.6	22.7	17.89	16.61	15.78	16.76	110.7	110.1	101.3	107.4
NP + $Ca(OH)_2$	25.0	28.3	27.0	26.8	12.70	12.64	12.70	12.68	102.9	107.2	103.2	104.4
N + $Ca(OH)_2$	45.2	35.2	43.6	41.3	8.58	7.42	7.11	7.70	92.0	88.3	89.6	90.0
P + $Ca(OH)_2$	51.3	54.9	44.6	50.3	11.44	12.99	12.74	12.39	103.2	106.7	106.1	105.3
M + $Ca(OH)_2$	56.4	47.6	53.6	52.5	17.40	15.53	16.22	16.38	112.3	115.7	111.7	113.2
Check	74.1	73.9	78.9	75.6	5.78	7.81	8.81	7.47	101.6	97.6	102.4	100.5

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[a] All figures represent averages of three replications.

[b] Based on stand of 26, 136 per acre.

lower than in 1939. The stand of beets was just about the same in both years.

Sugar beets, grown in the plots treated with $Ca(OH)_2$, showed, in both years, a very high amount of seedling disease, low yields, and relatively low stands. When identical plots, treated with $Ca(OH)_2$ are compared with those receiving no treatment, the 1939 results show that sugar beets planted in the plots treated with lime had considerably less seedling disease than those in non-treated plots, but in 1940 there was very little difference in the percentage of such disease in all plots. The yield and stand of beets, however, were about the same in both years for the plots treated and non-treated with lime.

Lime was added with some of the soil amendments, because the heavy clay-loam soil of the Huntley area is essentially free of carbonates. The lime was applied to improve the physical conditions of the soil, thus making it more porous, giving better aeration, and facilitating greater microbiological activity, including nitrificaton. Lime added to the soil also may serve as a readily available source of calcium.

The check plots showed the highest percentage of seedling disease (av. 75.4 per cent), lowest yields (av. 6.9 tons), and stands of all plots used in both years. The percentage of seedling disease in the check was several times higher than in the plots with the best soil treatments.

Those plots receiving the most nearly complete soil amendment each year showed a tendency toward a decrease in percentage of seedling disease and an increase in yield in 1940, as compared with 1939. The check plots, the plots treated with $Ca(OH)_2$ only, and those given a single soil amendment showed, in general, an increase in seedling disease. Contrary to expectation, there was a slightly greater increase in yield and in stand in 1940 than in 1939. There undoubtedly are present two parallel and hardly separable effects of fertilizers on sugar beets, one on the control of the seedling disease of beets and the other on the yield.

In the type of root rots occurring in Montana, although many of the diseased seedlings recover, there usually is a high mortality. Damage to the crop, therefore, results not only from reduced stand, but to some extent from retarded growth of plants of low vigor. In spite of a comparatively good stand of beets in the plots showing an abundance of seedling disease, the yields of these plots were poor because of small beets.

DISCUSSION

An analysis of the results of these experiments indicates that certain conditions characterizing these heavy Montana soils are responsible for the occurrence of these root rots of sugar-beet seedlings. It is evident from the 2 years' results that the plots receiving the most complete and balanced soil amendments produced the minimum of diseased seedlings, the largest yields, and, comparatively, the best stands. The fact that the seedling root rots can be controlled by an application of a complete and balanced fertilization shows that the disease factor itself is not the most important, but that the soil conditions are of first significance. The only exception seems to be in the case of the plots treated with manure, and with manure and phosphates, which, in spite of a considerable showing of seedling disease, yielded well, especially in 1940. This apparently contradictory situation may possibly be explained on the basis that sugar beets require a generous amount of easily available nitrogen in their early stage of development. Manure, with potentially a large supply of nitrogen, may have only a small part of it in nitrate form at the time of the early development of the beets. Soil temperatures are not then favorable for nitrification; so there may be a deficiency in nitrates, even in the soils sufficiently fertilized with manure. Later in the season, with increase in nitrification, the beets, even if affected with root rots, will have a better chance of survival and a more normal development than beets grown in soils of low fertility.

It is well known that sugar beets depend for their growth on a large amount of easily available nutrients; that the feeding area of beet roots in their early stages of development, both horizontally and vertically, is rather limited (10), that beet seedlings, up to the 6-leaf stage are very susceptible to seedling diseases, and that they later become more resistant (4, 5). This resistance, in a broadly interpreted sense of the term, is possibly attributable to the fact that as soon as the sugar beets increase their root systems and, consequently, their feeding areas, more nutrients are absorbed. In well-fertilized soils there are adequate nutrients for the young beets, consequently, they are resistant to root rots at a very early stage as compared with beets grown in soils of low fertility. Indications are that development of black root of sugar beets is facilitated by soils of low fertility with a small amount of easily available nutrients. Weather and many other factors also may influence these diseases, but it seems that soil nutritional factors have the greatest effect on the occurrence of certain soil-borne diseases of plants, a fact emphasized by several investigators (1, 2, 8, 9).

Furnishing readily available plant food to the young sugar beet while it is becoming established and its root system is small, appears to be a significant factor in making the plant more resistant to the invasion by some soil or seed-borne pathogens. Proper and complete soil fertilization also may affect the root-rot organism or organisms of young sugar beets. Sanford, in his studies of *Rhizoctonia solani* (6), reports that there is evidence indicating that conditions favorable to marked vegetative growth of the pathogen tend to depress its virulence.

It is quite possible that the pathogenicity of the root-rot fungus or fungi is noticeably less in soils well supplied with nutrients than in those poorly supplied. It is also possible that soil containing a considerable amount of nutrients stimulates the development of saprophytic soil organisms, which, through competition, may reduce the number of parasitic organisms in the soil.

SUMMARY

Seed treatments alone were found to be only slightly beneficial in controlling seedling diseases of sugar beets.

Soil treatments, regardless of seed treatments, proved highly important to the control of seedling diseases of sugar beets.

Plots with the most complete soil treatments, namely NPM, $\frac{N}{2}$PM, $\frac{N}{2}$P, NPM + $Ca(OH)_2$ and NP + $Ca(OH)_2$ produced the minimum of seedling disease, and the highest yields and stands.

Plots treated with NP, $\frac{N}{3}$ P, and MP had fairly good yields and stands, yet had considerable amounts of seedling diseases.

The plots with unbalanced soil amendments (N, P and M), all showed a high amount of seedling disease; the yields and stands also were poor, except for plots treated with manure.

Check plots and those treated only with $Ca(OH)_2$ had the highest amounts of seedling diseases and poorest yields and stands.

Seedling diseases of sugar beets in heavy, irrigated Montana soils can be efficiently controlled and at the same time good stands and high yields of sugar beets obtained by creating conditions that will promote a rapid and healthful development of young sugar beets through sufficient and balanced fertilization and improvement of the physical condition of the soil.

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NOTES ON CLITOCYBE ROOT ROT OF BANANAS AND OTHER PLANTS IN FLORIDA

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This paper presents an account of the occurrence of Clitocybe root rot of bananas in Florida, an abstract of which was published in 1932 (6), and also records the extreme destructiveness of this disease on other exotic plants, chiefly subtropical, on the same property on which it proved so destructive to bananas.

On January 24, 1931, the writer visited a property at Artesia, Brevard County, Florida, to investigate a suspected case of Clitocybe root rot of banana plants. An inspector of the State Plant Board had previously collected and sent for diagnosis the base of one plant to the Florida Agricultural Experiment Station at Gainesville. This property is located between Cocoa Beach and Canaveral on the barrier reef along the East Coast. The soil is Palm Beach sand, and, prior to clearing, was covered with a dense hammock forest in which live oak trees were predominant. Although the land had been cleared for several years, many oak roots had been left in it and the situation was a very favorable one for the development of the Clitocybe root-rot fungus.

Several banana plants of 3 varieties were found attacked by the disease in question. An examination of the interior tissues of the bases of the attacked plants revealed an unusually luxuriant development of the characteristic whitish mats of mycelium and rhizomorphs of the fungus permeating not only the fibrous tissues of the corm but also the base of the pseudostem (Figs. 1 and 2). The remains of an old cluster of the toadstools of *Clitocybe tabescens* (Scop.) Bres. were found at the base of one of the attacked plants.

Peeling off the outer dead leaf bases of attacked plants immediately disclosed the extent of the invasion of the bases of the pseudostems by the fungus. All the attacked plants examined were characterized by a watery, dark-brown zone involving the basal portion invaded by the whitish rhizomorphs and extending upward for some distance beyond the general level of the invaded portion in a series of irregular streaks (Fig. 2). This basal discoloration of the pseudostem ranged from a few inches to about a foot in height.

In dissecting attacked plants by carefully peeling off the fleshy leaf bases many varying and intricate patterns of rhizomorph formation were apparent. These not only penetrated the spongy, septate tissues of the succulent leaf bases but frequently grew out through the surface and developed between the compact concentric arrangement of leaf bases composing the pseudostem. They ranged from numerous, more or less branching thread-

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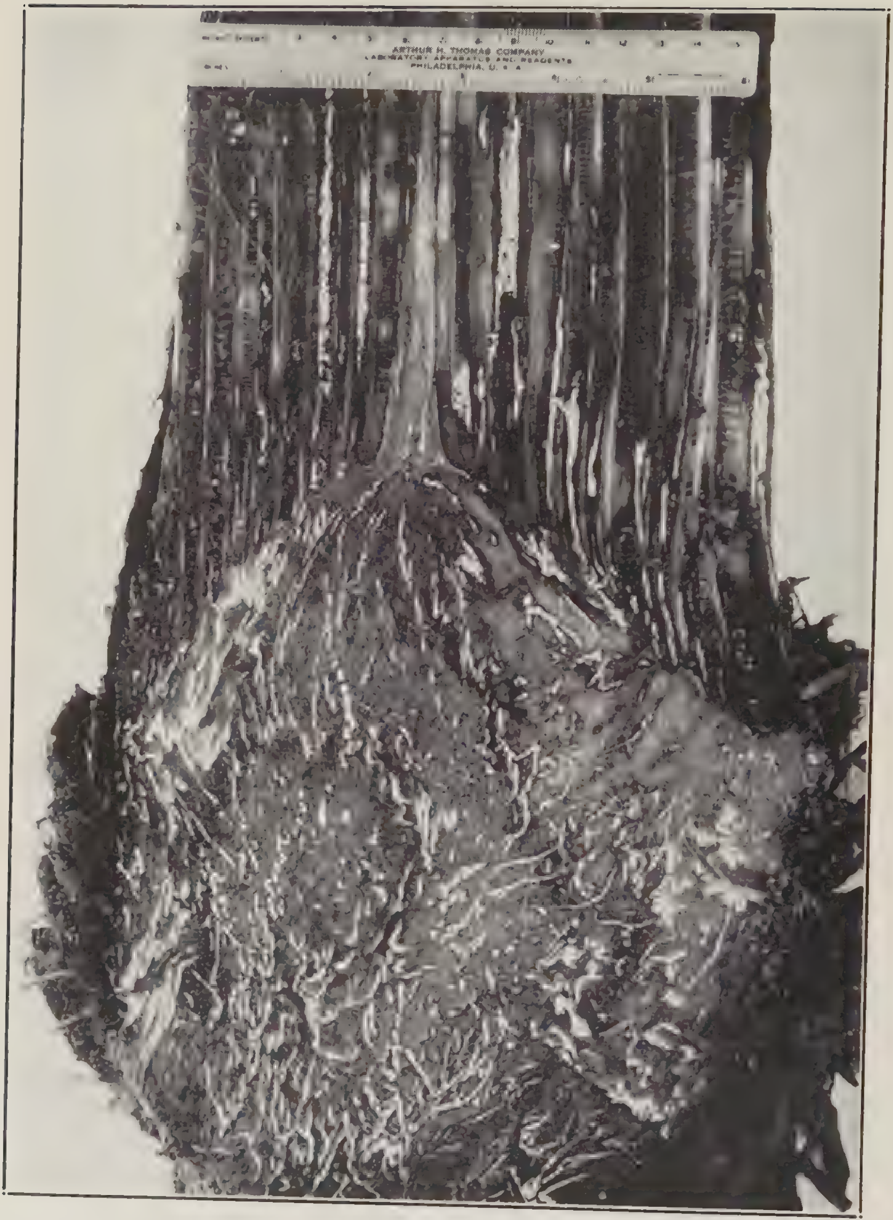
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FIG. 1. Longitudinal section through corm and base of pseudostem of banana plant recently killed by Clitocybe root rot, showing whitish mycelial mats and rhizomorphs irregularly distributed throughout the tissues.

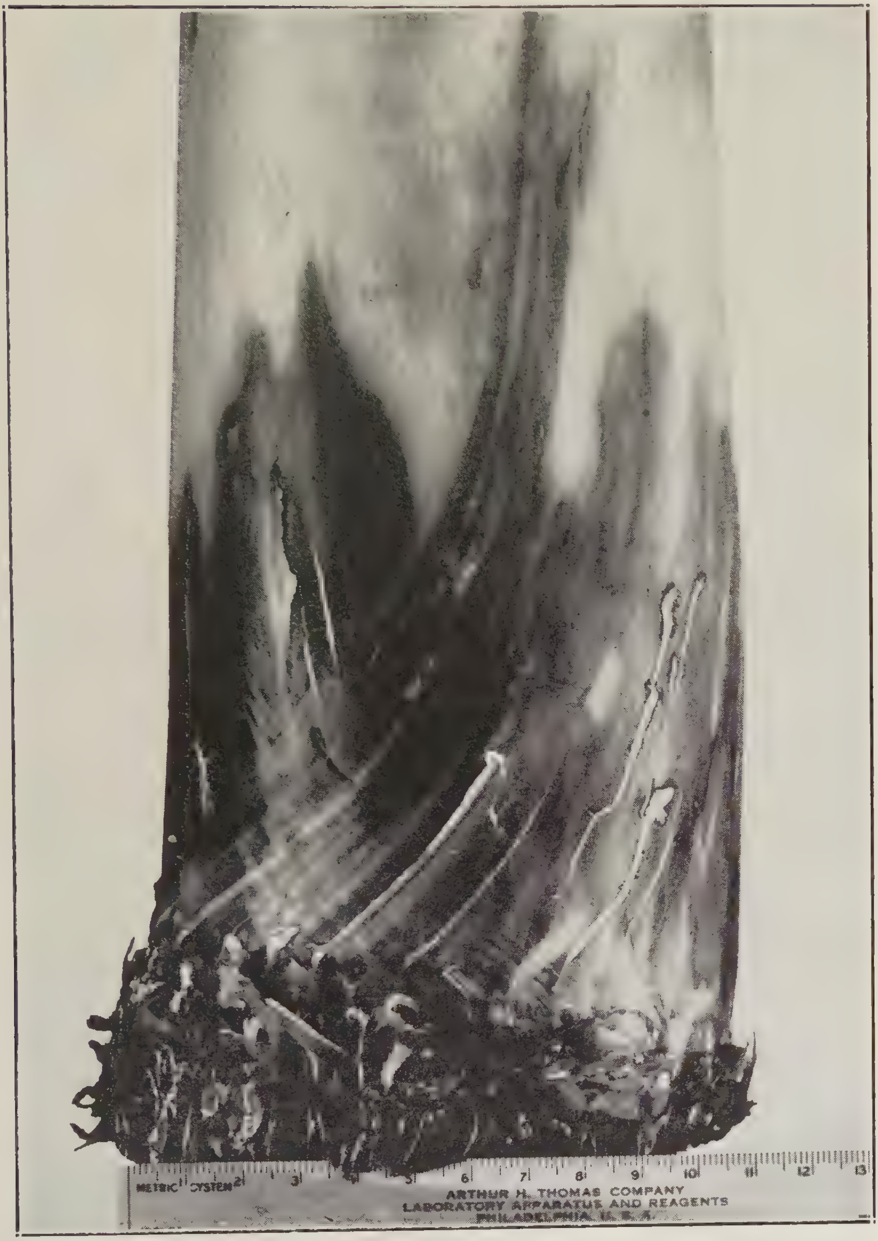
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FIG. 2. Base of pseudostem of banana plant recently killed by Clitocybe root rot, with outer leaf bases peeled off, showing the watery, dark-brown zone characterizing the portion invaded by whitish rhizomorphs of the fungus and extending upward in a series of irregular streaks.

like rhizomorphs to sometimes greatly broadened, irregular, thallus-like, whitish rhizomorphic sheets with the margins dividing into a series of simple or branching, thread-like rhizomorphs (Fig. 3, A). Sometimes the broad thallus-like rhizomorphs were irregularly lobed and, particularly where of recent growth, had developed little or no thread-like branching from the margins (Fig. 3, B). The broad, thallus-like type of rhizomorphs sometimes exhibited a peculiar perforate character, which is apparent in figure 3. The reason for this character is unknown, but it has been observed frequently in mycelial sheets of *C. tabescens* developed between the bark and wood of a wide variety of hosts, including both hardwood and coniferous trees, and appears to be a characteristic feature of this fungus. The rhizomorphic strands and sheets thus developed in and between the banana leaf bases were essentially counterparts of the same structures commonly produced in luxuriantly growing flask cultures of the fungus. However, according to extensive observations over a period of several years in various parts of Florida, the black, rounded or flattened, cortical and hypogeal rhizomorphs, so commonly produced by the closely related *Armillaria mellea*, are not produced by *C. tabescens*.

Pure cultures of *C. tabescens* were isolated subsequently from the interior tissues of each of two different plants. Subcultures of these were carried to fruition in comparison with isolations of this fungus from various other plants attacked by Clitocybe root rot, including some from the same property.

Upon transmitting to Gainesville specimens and the results of his findings, the writer was informed that a specimen of banana plant with the same trouble had been received from Nocatee, DeSoto County, Florida, on December 12, 1929, though it had not been so identified at the time. A portion of the latter specimen had been preserved, so that it was available for comparison. Unfortunately, however, no record was made of the name of the person who sent this specimen; otherwise a special effort would have been made to visit this property. On May 18, 1934, Mr. Erdman West, Mycologist of the Florida Agricultural Experiment Station, collected at Gainesville a specimen of Orinoco or "horse" banana, which he found attacked by Clitocybe root rot. This occurred in the yard of an apartment house where loquats and poinsettias in the same vicinity had died previously, presumably from the same trouble.

On September 1, 1931, Clitocybe root rot was found to be spreading on the banana plants at Artesia. A number of attacked plants were dug up for examination and all exhibited the same characteristic symptoms as those examined in January. A miniature cluster of young sporophores was found developing under the outer leaf base at the base of one plant. This was an exact counterpart of those that had developed on many occasions in cultures of the fungus from various sources. The stump of this plant was transported to Cocoa to see if further growth of the sporophores could be induced, but they shriveled after a few days.

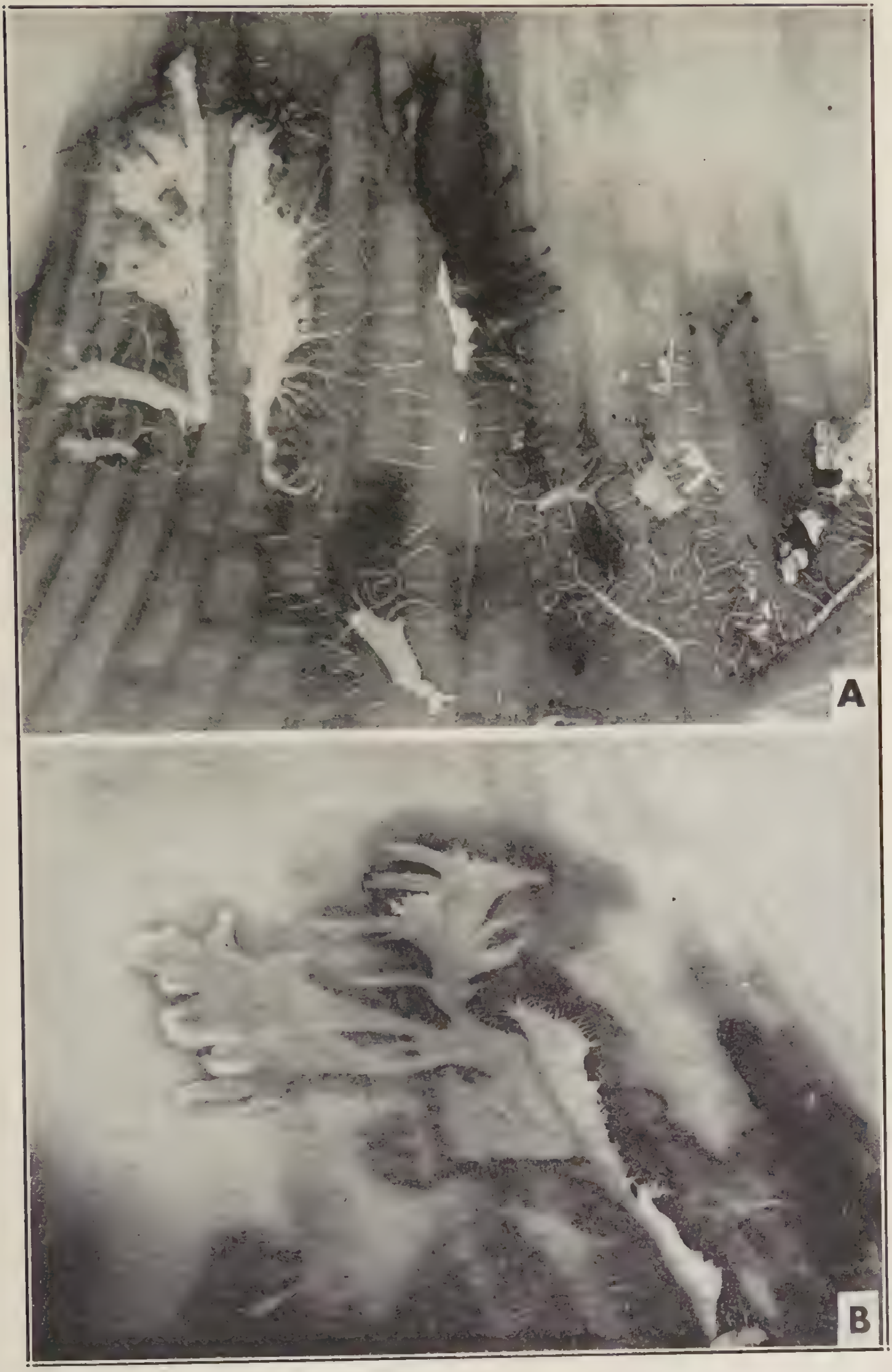

FIG. 3. Inner surfaces of banana leaf bases, flattened, showing various types of imbedded and superficial rhizomorphs. A. Rhizomorphs ranging from thread-like to thallus-like formations with a perforate character, the strip torn through broad one at left adhering to outer surface of next leaf base. B. Irregular branching, thallus-like rhizomorph with perforate character developed within the septate tissues, showing dark-brown, watery zone developing in advance of invasion. Natural size.

In checking over the banana mortality with the grower, it was estimated that he had already lost one stool of the Cavendish or Chinese (*Musa cavendishii* Lamb), 4 of the Hart's Choice or Lady Finger (*M. sapientum*, var. *champa* Baker), and 4 or 5 of the Orinoco or "horse" banana (*M. paradisiaca* L.). These were all planted close together. The grower stated that he also had lost a stool of the Red Jamaica variety, planted some distance away; but, since this had been removed and destroyed, it was impossible to verify this instance as an additional case of root rot. An investigation of the mode of spread of the disease showed that when plants became infected the fungus gradually spread through the corms and up first one shoot and then another, even attacking shoots but 2 to 3 feet high, and eventually killing the entire stool. Cultures of *C. tabescens* were isolated from the interior tissues of a dead young sucker of Hart's Choice banana and carried to fruition.

Unfortunately, banana plants on which inoculation experiments could be performed with pure cultures of *Clitocybe tabescens* associated with the corm rot here reported were not available. However, extensive experience with the destructive nature of this widely distributed parasite, both on the property at Artesia and those at numerous other points in Florida, leave no doubt about this fungus being the cause of the disease reported. In fact, the writer (7, 8) has produced infection and death of Australian pine (*Casuarina equisetifolia*) trees in Florida through inoculations with pure cultures of this fungus, and Plakidas (5) has done likewise with pineapple pear trees in Louisiana.

Since the observations here reported were made, the occurrence of a corm rot of bananas, due to *Clitocybe* sp., has been reported from New South Wales, Australia. In 1934, Noble (3) mentioned dry rot (*Clitocybe* sp.) as one of the diseases affecting Cavendish bananas in newly cleared lands, though in a previous report (2) he stated that "Banana root rot (*Armillaria mellea*) was reported in some areas." In a list of plant diseases recorded in New South Wales, prepared by him and others (4, p. 27), both diseases are listed as occurring on banana. *Armillaria mellea* is followed by the notation "Root Rot. 1917. N. Coast," while *Clitocybe* sp. is followed by the notation "Corm Rot. 1929. N. Coast." Whether both root rots of banana occur or the disease was first attributed to *Armillaria mellea* and later to *Clitocybe* sp. is not clear.

A brief account of corm rot of bananas was given in the following year by Magee and Eastwood (1), who state that it has been known for some years but has become of greater importance with the extension of banana planting to hardwood forest lands. The disease is said to be encountered but rarely in scrub lands (brushwoods), but, in forest and bastard scrub country, up to 300 to 400 stools have been lost at times in a 10-acre plantation. Corm rot is considered to be primarily a disease of new plantations on recently cleared land. The Australian disease results in a "dry brown corm rot, with white fungous threads interwoven throughout." The symptoms of the plant decline are described and appear to agree closely with those of the banana root rot here reported from Florida. A species of *Clitocybe* is said to be one of the most commonly associated parasites, but, unfortunately, the species was not determined—a point of vital interest. So far as the writer knows, *C. tabescens* has never been reported as occurring in Australia.

The owner of the property at Artesia, who had an ornamental nursery and a collection of miscellaneous trees, including several rare subtropical ones, proved to have had considerable experience with plants. Upon being informed as to the cause and nature of the disease attacking his bananas on the occasion of the writer's first visit in January, 1931, he mentioned that he had lost an apple and an apricot tree some time before, due, he thought, to borers. The apple tree was 5 years old from the seed and the apricot tree was a budded one purchased from a nurseryman 4 years previously. They were near each other and about 200 feet from the attacked banana plants. Upon examination, both showed the characteristic mycelial sheets of the Clitocybe root rot fungus between the bark and the wood at the base and were dug up for cultures. *C. tabescens* was isolated subsequently from the apple tree and carried to fruition, but was not secured from the apricot tree, as it was pretty well desiccated. The writer was then informed that a guava (*Psidium guajava*) and a Java-plum (*Eugenia jambolana*) also had died. The latter had been cut off, but the stump remained. Upon examination, it was found that both trees were attacked by Clitocybe root rot.

On the occasion of the writer's second visit in September, 1931, the disease had spread to the one remaining trunk of the guava clump that was found partly dead in January. This appeared quite healthy and had a fair crop of fruit nearing maturity. However, examination of the base revealed the characteristic development of the Clitocybe root-rot fungus under the bark, encircling the trunk up to a point about 18 inches high, where the trunk forked, and continuing up one division to a point 2 feet high. The invaded bark at the upper limit of the lesion was characterized by a slight sunken condition, which demarked the progress of the disease. While the life of this trunk did not appear to be affected, the top wilted and died shortly thereafter.

A large specimen of the trumpet-tree (*Cecropia palmata*) also was found attacked by Clitocybe root rot. The top was in good condition, but the fungus had invaded the bark of the trunk and the roots at one side of the base. The grower had recently cut away the dead bark at the base and had removed a large dead lateral root that remained lying by the tree. This root had an abundant development of mycelium under the bark, resulting in a soft white decay of the delignifying type. Another smaller specimen of trumpet-tree, planted in another portion of the property, also was found to have been killed by root rot.

A magnificent example of rose-apple (*Eugenia jambos*) also was found to be attacked by Clitocybe root rot. A small bark lesion occurred at one side of the base of the trunk and the removal of the soil at this point disclosed a large dead lateral root. The bark of both contained a luxuriant development of mycelium.

The grower stated that about 2 months previously he had removed a cherimoya (*Annona cherimola*) and a soursop or guanabana (*Annona muricata*), both of which apparently had died from the same trouble. The places where these trees had been dug out were between 7 and 8 feet apart and both were on a 20-foot radius from the infected rose-apple. The grower recalled that about 2 years before he had removed another cherimoya tree, growing about 8 feet from the other. It was learned that the trunks of both the cherimoya and the soursop trees, which had been removed 2 months before, had been dumped into a nearby ditch. The abundant development of the characteristic mycelial sheets of the Clitocybe root-rot fungus, found under the bark of the roots of both these trees, was convincing evidence that they also were victims of this destructive root-rot fungus. At another point on the property a third cherimoya tree, which had died recently from the same trouble, was found. This tree had a bark lesion all around the base and, when dug up, the bark of this lesion and that of the entire root system was found invaded by mycelium. *C. tabescens* was isolated from the roots of this tree and also from a rose bouquet (*Dombeya punctata*) bush that had died from root rot on a neighboring property and carried to fruition in each case. Before leaving this extremely interesting property a young Abundance plum, a large Woodland Margaret rose on its own roots, and a hibiscus (*H. rosa-sinensis*) bush also were found to have succumbed to Clitocybe root rot.

On August 3, 1932, another visit was made to this property and several new plants were found to be attacked by Clitocybe root rot. Among these may be mentioned a Mexican guava (*Psidium molle*) and a Java-plum (*Eugenia jambolana*), both of which had been dead a few months, as well as a large Catalonian jasmine (*Jasminum grandiflorum*) vine that recently had shriveled and died. A 7-inch Carolina laurel cherry (*Laurocerasus caroliniana*) but a foot away from this jasmine next attracted the writer's attention by reason of a profuse basal gumming that extended about two-thirds around the trunk. The dead area of bark, apparent on one side, showed the development of the Clitocybe root-rot fungus. A few feet distant another Carolina laurel cherry of the same size was found with identical symptoms. The root rot had not progressed sufficiently in either case to affect the tops of these trees. In another part of this small property a sapodilla (*Achras sapota*) was found to have shriveled without loss of the foliage at about the time the fruit was maturing. This tree was dug up for examination and nearly all the larger roots were completely invaded by the mycelium of the Clitocybe root-rot fungus.

A physic nut (*Jatropha curcas*) also was found completely girdled by the same fungus, with the bark dead up to an average height of a foot, at which point a pronounced callus formation had developed at the lower margin of the bark remaining alive. The top of this tree was still living but had comparatively few leaves remaining. The grower reluctantly permitted this tree to be dug up. It was found to have a twin tap root, one division of which had a luxuriant development of the Clitocybe root-rot fungus under the bark. A few concentric and occasional radial cracks occurred in the wood of this root, into which sheets of mycelium had grown. Where the side of the other division of the taproot was in contact with this dead one the bark was found to have become infected—a typical case of the spread of this root disease by contact of a sound root with an infected one. Some of the lateral roots also were found attacked by the root-rot fungus. Pure cultures of *C. tabescens*, with the usual abundant production of whitish rhizomorphs, were obtained from the roots of both this and the sapodilla tree and carried to fruition.

On April 30, 1941, the grower stated that the disease had attacked a litchee (*Litchi chinensis*) about 3 or 4 years previously, but that he had treated it and the tree had since borne good crops of fruit. He further stated that he had found that the recommended procedure of exposing the root crowns of attacked plants to aeration and drying had proved effective in arresting the progress of the disease, and that with this in conjunction with surgical treatment he had been able to save a number of trees attacked by root rot, provided the disease was detected and treatment administered before it had progressed too far.

The high degree of mortality in that portion of this small property devoted chiefly to subtropical plants was attributable largely to the practice of close planting, allowing the diseased trees to remain frequently long after they had died, and incomplete removal of the roots when the trees were finally dug up, thereby increasing the number of centers of infection. As a result, most of the plants enumerated were attacked or killed by the time they had attained an age of from 4 to 7 years. Doubtless a number of other new hosts for Clitocybe root rot might have been determined had the writer continued to have access to this property after 1932. However, circumstances arose to prohibit further inspection of the plantings for the purpose in which he was interested.

SUMMARY

A mushrooom or toadstool root rot of bananas occurring in Florida, caused by *Clitocybe tabescens*, which has been found to be of widespread occurrence in this State and frequently very destructive to a large variety of native trees, fruit trees, ornamental trees, shrubs and vines, is reported.

The symptoms, which are described and illustrated in detail, agree closely with those of a corm rot of bananas reported as becoming increasingly prevalent in New South Wales, Australia, as plantings are extended to newly cleared hardwood forest land, and attributed to an undetermined species of *Clitocybe*.

Notes are also given on the widespread loss of a number of trees and

other plants, mostly exotic and including a number of uncommon subtropical ones, from attack by *Clitocybe tabescens* in the same property where this disease proved so destructive to bananas.

COCOA, FLORIDA.

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THE FUNGICIDAL AND PHYTOCIDAL PROPERTIES OF SOME COPPER XANTHATES

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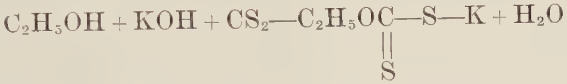
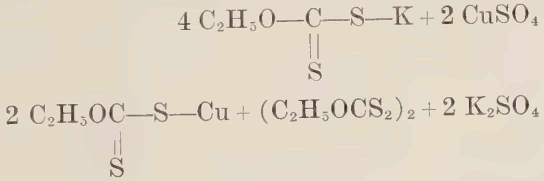
The copper xanthates are of interest to plant pathologists working with fungicides because they contain both copper and sulphur and are nearly insoluble in water. They are the copper salts of the theoretical xanthic acids $R \cdot OCS_2H$, in which R represents an alkyl organic radical. In water suspensions only small amounts of copper or sulphur appear to be available at any one moment. Our study of their properties was the result of a search for less injurious substitutes for the common orchard fungicides, Bordeaux mixture, sulphur, and lime-sulphur, all of which may cause injury to fruit trees under certain conditions.

The soluble xanthates are made by combining equimolecular quantities of an alkali or alkaline earth metal hydroxide, carbon disulphide, and an alcohol or a compound containing an alkyl hydroxyl grouping. The following example indicates the reactions concerned in the formation of potassium ethyl xanthate:



$$C_2H_5OH + KOH + CS_2 — C_2H_5O\underset{\underset{S}{\|}}{C}—S—K + H_2O$$

Cuprous xanthates may be made by the treatment of a solution of an alkali metal xanthate with a solution of a copper salt. Di-xanthogens, also, are produced in the resultant reaction and these were removed from the insoluble precipitates by treatment with acetone.



$$4\ C_2H_5O—\underset{\underset{S}{\|}}{C}—S—K + 2\ CuSO_4$$

$$2\ C_2H_5O\underset{\underset{S}{\|}}{C}—S—Cu + (C_2H_5OCS_2)_2 + 2\ K_2SO_4$$

For the experiments reported in this paper the cuprous xanthates of the lower alcohols (methyl, ethyl, propyl, butyl, isoamyl),[1] and some of their isomers[2] were made by treating solutions of cupric sulphate with the potassium xanthates of the respective alcohols. The cuprous xanthates precipitate out as finely divided yellowish powders, not wettable in water. Copper ethyl xanthate and copper isoamyl xanthate were prepared for us in larger lots for field trials through the courtesy of the Monsanto Chemical Company, of St. Louis, Mo. The copper content of the samples (Table 1) compared to the theoretical copper content indicated a high degree of purity.

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[1] A material sold as isoamyl alcohol was used. The exact content of this compound it not known.

[2] Carter, R. H. Insecticidal and fungicidal compositions. U. S. Patent No. 2150759. U. S. Patent Office, Washington, D. C. 1939.

TABLE 1.—*Copper content of various copper xanthates*

Compound	Percentage copper theoretical	Percentage copper found
Cuprous methyl xanthate	37.2	35.7
" " "		37.4
Cuprous ethyl xanthate	34.4	32.0
" " " commercial[a]	......	38.6
Cuprous N propyl xanthate	31.6	31.1
" iso " "		33.1
Cuprous N butyl xanthate	29.8	29.7
" " " "		26.2
Cuprous isoamyl xanthate	28.0	27.3
" " " "	....	29.3
" " " " commercial[a]	....	28.8

[a] Obtained through courtesy of the Monsanto Chemical Co., St. Louis, Mo.

Table 2 shows the solubility of 5 of these compounds produced in our laboratories and known to be pure. All of the solutions were found to react with chromotropic reagent, a sensitive indicator for dilute concentrations of ionized copper[3] and none gave indications of SH ions when treated with 5 per cent nitroprusside solutions.[4] All, and especially the solutions of the methyl, propyl, and butyl copper xanthates, possessed odors indicating sulphur affinities.

The laboratory tests were conducted according to the methods outlined by Goldsworthy and Green[5] and consist, briefly, in subjecting conidia of *Sclerotinia fructicola* (Wint.) Rehm. (peach brown-rot pathogen) and *Glomerella cingulata* (Ston.) Spaul. and Schrenk (apple bitter-rot pathogen) to 24-hour perfusions with saturated water solutions of the chemicals, and to residues of the chemicals remaining on sprayed glass cover slips after weathering in an apple orchard. Phytocidal tests were conducted on bean, *Phaseolus vulgaris* L. var. Red Kidney, and on apple, *Malus sylvestris* Mill. var. Starking Delicious.

Copper methyl, propyl, and butyl xanthates possess such a penetrating and disagreeable odor that they were not used in field residue tests.

The perfusion and phytocidal tests were conducted in the laboratory and greenhouse with the copper methyl, ethyl, propyl, butyl, and isoamyl xanthates. Table 2 shows the fungicidal value of the saturated solutions of the various copper xanthates against the conidia of *Sclerotinia fructicola* and *Glomerella cingulata*.

All of the saturated solutions were found to be toxic in varying degrees to the conidia of *Sclerotinia fructicola*. Of the 5 tested, copper ethyl

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[3] Goldsworthy, M. C., and E. L. Green. Availability of the copper of Bordeaux mixture residues and its absorption by the conidia of *Sclerotinia fructicola*. Jour. Agr. Res. [U.S.] 52: 517–535. 1936.

[4] ————. The fungicidal action of liquid lime-sulfur. Phytopath. 18: 355–360. 1928.

[5] Goldsworthy, M. C., and E. L. Green. Effect of low concentrations of copper on germination and growth of conidia of *Sclerotinia fructicola* and *Glomerella cingulata*. Jour. Agr. Res. [U.S.] 56: 489–506. 1938.

TABLE 2.—*Fungicidal value of saturated aqueous solutions of copper xanthates applied to the condia of S. fructicola and G. cingulata*

Material	Solubility in water in p.p.m.[b]	Hours perfused	Effect on the condia of *S. fructicola*, 7 days old, grown at 75° F.	Sub-growth[a]	Effect on the conidia of *G. cingulata* 5 days old grown at 75° F.	Sub-growth[a]	Growth in controls	
							S. fruti-cola conidia	*G. cingu lata* conidia
				Per cent		*Per cent*	*Per cent*	*Per cent*
Copper methyl xanthate	0.7	6	Few cells plasmolyzed	32	No germination; no apparent injury	82	100	96
		24	All appear plasmolyzed	12	82 per cent germinated	86	100	100
Copper ethyl xanthate	1.78	6	Many appear plasmolyzed	12	No germination	52	100	96
		24	All appear plasmolyzed	0	Mostly all plasmolyzed	11	100	100
Copper propyl xanthate	0.75	6	No apparent injury	86	No apparent injury	92	100	82
		24	Some plasmolysis	16	Some cells appear injured	74	100	100
Copper butyl xanthate	0.62	6	All appear plasmolyzed	12	Many injured	62	100	96
		24	All plasmolyzed	0	“ “	48	100	100
Copper iso-amyl xanthate	1.18	6	Some plasmolysis	91	No apparent injury	84	100	82
		24	Many cells injured	12	Some plasmolysis	62	100	100

[a] Perfused conidia transferred after 24 hours to potato-hard-agar and incubated for 24 hours and observed for continued growth.

[b] Parts Cu per million in distilled water.

xanthate and copper butyl xanthate showed the greatest fungicidal value at the end of 6 hours of perfusion; at the end of 24 hours, both prevented germination entirely. Methyl, propyl, and isoamyl xanthates showed the least fungicidal value to the conidia of *S. fructicola.* None of the xanthates was completely effective against the conidia of *Glomerella cingulata,* but all showed some degree of fungicidal value. Copper ethyl xanthate proved to be the most potent of the 5 used against this organism. It is not known whether the fungicidal value of these solutions was due entirely to available copper or to dissolved sulphur. The fungicidal values obtained appear to indicate that they are attributable to the presence of available copper rather than to available sulphur. The varying responses of the conidia of both species are indicative of the presence of copper values. If the results were due to the presence of soluble sulphur it appears very unlikely that the conidia of *G. cingulata* would have responded to the treatments. The data also appear to support the theory that only a portion of the soluble copper was available for the reactions, since none of the actual values approached the theoretical values for the effect of copper.

TABLE 3.—*Fungicidal value of various concentrations of soluble copper, as copper sulphate, applied to the conidia of Sclerotina fructicola and Glomerella cingulata at 74° F.*

Copper concentration	Hours perfused	Effect on the conidia of *S. fructicola,* 7 days old	Subsequent growth	Effect on the conidia of *G. cingulata,* 5 days old	Subsequent growth
			Per cent		*Per cent*
0.25 p.p.m.	6	Some slight injury noted	90	No apparent injury	100
	24	Apparent injury to some	56	" " "	100
0.50 p.p.m.	6	Few have germinated; few appear injured	48	" " "	68
	24	All appear injured	Trace	Many appear injured	42
0.75 p.p.m.	6	Some apparent injury, no germination	35	Few appear injured	80
	24	All appear injured	0	All appear injured	Trace
1.00 p.p.m.	6	Trace of germination; some appear injured	46	Few " "	72
	24	All appear injured	0	All " "	Trace
1.50 p.p.m.	6	Few with short germ tubes; many appear injured	Trace	Few with short germ tubes; few appear injured	46
	24	All appear injured	0	All appear injured	Trace
2.00 p.p.m.	6	All appear injured	10	" " "	50
	24	" " "	0	" " "	0
3.00 p.p.m.	6	" " "	Trace	" " "	Trace
	24	" " "	0	" " "	0
Distilled H_2O	6	90 per cent budded	100	No germination	100
	24	100 per cent with short germ tubes	100	All have short germ tubes	100

Table 3 shows the effect of varying p.p.m. concentrations of soluble copper on the conidia of *Sclerotinia fructicola* and *Glomerella cingulata.*

Copper concentrations approximating 0.50 p.p.m. were toxic to the conidia of *S. fructicola* in the same degree as saturated solutions of copper ethyl and butyl xanthates, while copper concentrations of 0.25 p.p.m. were comparable with methyl, propyl, and amyl xanthates. Nearly all of the copper xanthates are about as toxic to the conidia of *G. cingulata* as a solution of copper containing approximately 0.50 p.p.m. (about the copper solubility of weathered 4–4–50 Bordeaux mixture). The saturated solution of copper ethyl xanthate was the most potent, and the indication was that if the toxicity was due to soluble copper the copper content was nearer 0.75 p.p.m. than 0.50 p.p.m., since it was more toxic than the known solution of copper containing 0.50 p.p.m.

The data from the residue tests are shown in table 4. The 2 xanthates, copper ethyl and isoamyl, were each combined with hydrated lime and bentonite at the rate of 2 lb. of xanthate, 4 lb. of hydrated lime, 2 lb. of bentonite, and $\frac{3}{8}$ lb. of soluble fish oil soap to 100 gal. of water. Each mixture was sprayed on thin cover glasses and allowed to dry thoroughly. The sprayed cover glasses were then hung up among the leaves of unsprayed apple trees and allowed to weather. Samples were collected on the 3rd, 7th, 15th, and 22nd day following the initial exposure and brought into the laboratory for testing. Drops of conidial suspensions were placed on the residues and the preparations were held at a high humidity to prevent drying out and at a temperature favoring germination. Observations of the direct effect of the spray residues were recorded after a 24-hour period, and then some of the conidia were transferred to a medium free of residue, to find out whether the conidia had been killed or merely inhibited. Bordeaux mixture, the fungicidal value of which is known, was used for comparison.

Before weathering, the residues of the copper xanthates showed slight toxicity to the conidia of *Sclerotinia fructicola,* but none to those of *Glomerella cingulata.* Lime residues also exhibit toxicity under these conditions, and probably the first day's effect was due to the lime. As time elapsed, both of the xanthates showed a progressive increase in toxicity against the conidia of *S. fructicola* but none against those of *G. cingulata.* During the same period the progress of toxicity with Bordeaux mixture was more rapid, as is characteristic of that material. From these data it appears certain that the residues of the copper xanthate mixtures are not so potent as those of Bordeaux, but that they may have possibilities against disease-producing organisms that are killed by more dilute copper solutions.

Phytocidal tests on the bean (*Phaseolus vulgaris* L. var. Red Kidney) and on apple (*Malus sylvestris* Niell. var. Starking), under average greenhouse conditions of either low or high humidity indicated that the copper xanthates were relatively safe, since no injuries were observed.

Copper ethyl and iso-amyl xanthates, combined with lime, bentonite, and lead arsenate (when needed as an insecticide), and wetted with soluble fish oil soap, were applied in field tests to apple varieties in 1937 to control

TABLE 4.—*Effect of weathering on the fungicidal value of spray residues composed of copper xanthates, hydrated lime and bentonite*

Material	Days weathered	Total precipitation	Effect on *Sclerotina fructicola* conidia, 7 days old, grown at 75° F.	Subsequent growth		Effect on *Glomerella cingulata* conidia, 5 days old, grown at 75° F.	Subsequent growth	
				Treated	Check		Treated	Check
Copper ethyl xanthate mixture 2–4–2–100[a]	0	0	Inhibited—no growth	78	100	78 per cent germinated	100	100
	3	0	Some inhibited; some plasmolyzed	30	100	Inhibited—no growth	100	100
	7	0	Some inhibited; some plasmolyzed	32	100	" " "	100	100
	15	0.07	All appear plasmolyzed	Few	100	" " "	100	100
	22	0.07	" " "	0	100	" " "	100	100
Copper isoamyl xanthate mixture 2–4–2–100[a]	0	0	Inhibited—no growth	82	100	68 per cent germinated	100	100
	3	0	Few appear injured; inhibited	62	100	All inhibited	100	100
	7	0	Some plasmolyzed; some inhibited	41	100	" "	100	100
	15	0.07	Many appear plasmolyzed; few inhibited	18	100	" "	100	100
	22	0.07	Mostly all plasmolyzed	6	100	" "	100	100
Bordeaux mixture 4–8–100[b]	0	0	All appear plasmolyzed	12	100	" "	100	100
	3	0	Mostly all appear plasmolyzed	Few	100	All appear injured or inhibited	46	100
	7	0	Mostly all appear injured	Few	100	All appear injured or inhibited	Few	100
	15	0.07	All appear plasmolyzed	0	100	All appear injured or inhibited	Few	100
	22	0.07	" " "	0	100	All appear injured or inhibited	Few	100

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[a] Two lb. of xanthate, 4 lb. of hydrated lime, 2 lb. of bentonite, 100 gal. of water.
[b] Four lb. of copper sulphate, 8 lb. of hydrated lime, 100 gal. of water.

the apple scab organism, *Venturia inaequalis* (Cke.) Aderh. The data from these tests are shown in table 5. The tests were conducted on a small scale, on 5-year-old Williams' Early Red, York Imperial, Rome Beauty and Starking apple trees at the U. S. Horticultural Station, Beltsville, Md. In table 5 the performance of the xanthates is compared with that of phenothiazine mixture in one case, lime-sulphur followed by copper-phosphate mixture in two cases, and with no treatment in another.

TABLE 5.—*Effect of copper ethyl xanthate and copper isoamyl xanthate on the control of apple scab and russet**

Variety	Treatment.	Total fruits	Scab		Russet	
			No.	*Per cent*	*No.*	*Per cent*
Williams' Early Red	Ethyl xanthate mixture[a]	240	6	2.5	18	7.5
	Isoamyl xanthate mixture	112	4	3.2	18	16.0
	Phenothiazine mixture[b]	334	2	Trace	40	12.0
York Imperial	Ethyl xanthate mixture	47	0	0.0	11	23.4
	Isoamyl xanthate mixture	27	0	0.0	3	11.1
	Lime-sulphur; copper-phosphate[c]	50	0	0.0	3	6.0
Rome Beauty	Ethyl xanthate mixture	11	0	0.0	4	36.4
	Isoamyl xanthate mixture	112	18	16.0	7	6.2
	Lime-sulphur; copper-phosphate mixture[c]	45	0	0.0	16	35.5
Starking	Ethyl xanthate mixture	228	10	4.3	35	15.4
	Isoamyl xanthate mixture	103	4	4.0	5	5.0
	No treatment	642	341	53.1	18	2.8

* Five applications: blossom, calyx, and 3 cover applications.

[a] Mixture = 2 lb. copper xanthate, 4 lb. of hydrated lime, 2 lb. of lead arsenate, and 2 lb. of bentonite to 100 gal. of spray fluid.

[b] " = 2 lb. phenothiazine, 4 lb. of hydrated lime, and 2 pounds of bentonite to 100 gal. of spray fluid.

[c] " = Lime-sulphur 1–40 at blossom, 1–50 at calyx, 1–75 at first and second cover applications. Copper phosphate 4 lb., hydrated lime 8 lb., bentonite 2 lb. to 100 gal. of spray fluid in third cover application. Lead arsenate 2–100 at calyx, first, second, and third covers.

The copper xanthates caused no injury that could be ascribed to their copper content. A type of injury developed, however, during the warm, humid periods, that greatly resembled that resulting from the use of arsenicals. On most varieties this injury affected mostly the margins of the leaves and caused but little damage. On the Williams' Early Red variety this injury also occurred in the intercostal regions of a few of the leaves.

Although the xanthates controlled apple scab fairly well under the circumstances of the tests, their performance, from a phytocidal and fungicidal standpoint, was inferior to that of the regular lime-sulphur and Bordeaux applications. They have no practical value as orchard spray materials at the present time.

SUMMARY AND CONCLUSIONS

The fungicidal value of the copper xanthates prepared from methyl, ethyl, propyl, butyl, and isoamyl alcohols were tested.

These materials are only slightly soluble in water. Saturated solutions contained very little available copper and apparently the copper and sulphur are combined so tightly that they are non-injurious to sprayed plants.

Copper ethyl and isoamyl xanthates were tested against the apple scab organism, *Venturia inaequalis*, in orchard experiments on 4 apple varieties. Although they reduced the number of scab infections, protection was not equal to that shown by the regular orchard treatments with lime-sulphur (early sprays) and copper phosphate mixture (late sprays).

In laboratory perfusion tests all of the xanthates tested proved toxic to some extent to the conidia of *Sclerotina fructicola*. The ethyl and butyl xanthates were the most toxic. None of the xanthates appeared to possess a high fungicidal value against the conidia of *Glomerella cingulata*. The toxicity exhibited by saturated solutions of the various xanthates against *S. fructicola* and *G. cingulata conidia* was of the same degree as that exhibited by a solution containing about 0.50 p.p.m. of copper.

Spray residues containing the ethyl and isoamyl xanthates were toxic to the conidia of *S. fructicola* but not to those of *G. cingulata*.

Applications of the xanthates on bean plants (*Phaseolus vulgaris* L. var. Red Kidney) and on apple (*Malus sylvestris* Niell. var. Starking), in the greenhouse caused no injury to leaves or stems at low and high humidities, respectively, and at average temperatures. In regular orchard treatments of apple varieties, copper ethyl and isoamyl xanthates appeared to promote arsenical injury when mixed with lead arsenate.

THE MECHANISM OF SPORE DISPERSAL IN PERONOSPORA TABACINA AND CERTAIN OTHER DOWNY MILDEW FUNGI[1]

J. A. PINCKARD[2]

(Accepted for publication August 4, 1941)

Sporangial release among the Peronosporales is usually described as a simple process brought about by the mechanical action of air currents or by rain. This concept assumes that the sporangia, upon reaching maturity, are broken off from their sterigmata-like attachments to the sporangiophore, more or less by accident. That this process is no mere accident was first suggested by de Bary (3), who wrote: "Where filiform sporophores rise free into the air, a further mechanical arrangement is found which greatly assists the shedding and scattering of the abscissed spores. It may be readily observed in the Hyphomycetes, in *Peronospora*, for example, *Phytophthora infestans*, and in the gonidiophores of *Peziza, Fuckeliana,* etc. The hyphae of these fungi are cylindrical in the moist and turgescent state, but collapse when dry *and especially* when the spores are ripe into a flat ribbon-like form, and the drier they are the more strongly do they become twisted round their own longitudinal axis. They are so highly hygroscopic that the slightest change in the humidity of the surrounding air, such, for instance, as may be caused by the breath of the observer, at once produces changes in their turgescence and torsion; the latter giving a twirling motion to the extremity of the gonidiophore and the ripe spores are thereby thrown off in every direction."

De Bary's interesting observations appear to have been either overlooked by most investigators or considered of little practical significance. Ingold (6), who reviewed the literature on spore discharge among land plants, quotes de Bary's statements in full, but adds: "It seems very likely that this kind of discharge may occur, but although I have examined the behavior of the conidiophores of *Peronospora parasitica* and *Botrytis cinerea* under conditions of rapidly changing humidity, I have been unable to convince myself that actual spore discharge is effected by the twirling movements of the conidiophore." Ingold concluded, therefore, that this form of spore discharge is of ". . . little or no biological importance. . . ." Weston (10), after studying exhaustively the production and dispersal of conidia in the Philippine *Sclerosporas* of maize stated, ". . . In the opinion of the writer these experiments and observations indicate that the conidia are forcibly thrown off from the sterigmata. . . ." Both de Bary and Weston, therefore, were of the opinion that mechanisms for forcible dispersal of sporangia occurred among certain Peronosporales.

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[1] Published with the approval of the Director of the Virginia Agricultural Experiment Station.

[2] Formerly Associate Plant Pathologist, Virginia Agricultural Experiment Station. The writer also wishes to express his gratitude to F. A. Wolf and to W. A. Whitney for helpful assistance.

During the course of extensive investigations (9) on the control of downy mildew of tobacco, caused by *Peronospora tabacina* Adam, the forcible ejection of sporangia of *P. tabacina* was observed to occur precisely as de Bary has described the process for *P. parasitica* (Pers.) Fr. The purpose of this report is to describe the hygroscopic mechanism of sporangial dispersal of *P. tabacina*, and similar fungi, and to discuss its importance in relation to the behavior of the downy mildew of tobacco (*Nicotiana tabacum* L.).

DEMONSTRATION OF THE HYGROSCOPIC MECHANISM

Several investigators (1, 11, 13) have described and illustrated the formation and production of the sporangia of *Peronospora tabacina*. Normally the sporangiophores and sporangia begin to form on the under surface of infected tobacco leaves a few hours before daybreak when temperature and atmospheric moisture are favorable. Numerous field observations (4) have shown that normal sporangial formation occurs within the range of 42° to 63° F., being most abundant at 56° F. Sparse sporangial formation will take place, however, both above and below these temperature limits (2). The effect of the water vapor pressure, or relative humidity, on sporangial formation, however, is pronounced. Armstrong and Sumner (2) have shown that sporulation is limited to the range from atmospheric saturation to slightly below the dew point. Weston (10), working with downy mildew of maize, observed that a film of moisture on the leaf seemed essential for normal sporulation. Sporangial formation by *P. tabacina* is inhibited, however, by the presence of a visible film of water on the leaf surface (Fig. 1).

If a leaf, bearing freshly-formed sporangiophores (Fig. 1), is mounted under a dissecting microscope, in such way as to show them in profile against a white background, and if the fruiting structures have not been subjected to drying between the time of maturation and examination, normal sporangiophores with their attached sporangia may be observed (Fig. 2, A). Then, if the normal, mature sporangiophores are watched, while slight drying begins, the entire crown of each fruiting structure, with its branchlets and sporangia, begins a counter-clockwise rotation. Each portion of a sporangiophore, lying between the branches, executes independent rotation. The degree of rotation appears to depend upon the length of the section between branches and the degree of desiccation.

Several complete twists occur in tall sporangiophores, in the portion extending up to the first branch. A lesser number of twists occurs between each successively shorter branch. As drying progresses, a twisting and bending motion is imparted to the sterigmata-like structure bearing the sporangia. If desiccation be discontinued, the movement ceases, and the rotation reverses itself, if air of a higher moisture content be supplied to the sporangiophores. Under conditions of delicate moisture balance, the breath of the observer is sufficient to bring about the above described movements. The net effect of these movements is, of course, the release of the mature sporangia. Figure 2, A, B, and C illustrates diagrammatically the sequence of sporangiophore movements in an atmosphere becoming progressively drier. Figure 2, D illustrates the reverse movement of the sporangiophore shown in figure 2, C, after saturated air has been re-introduced.

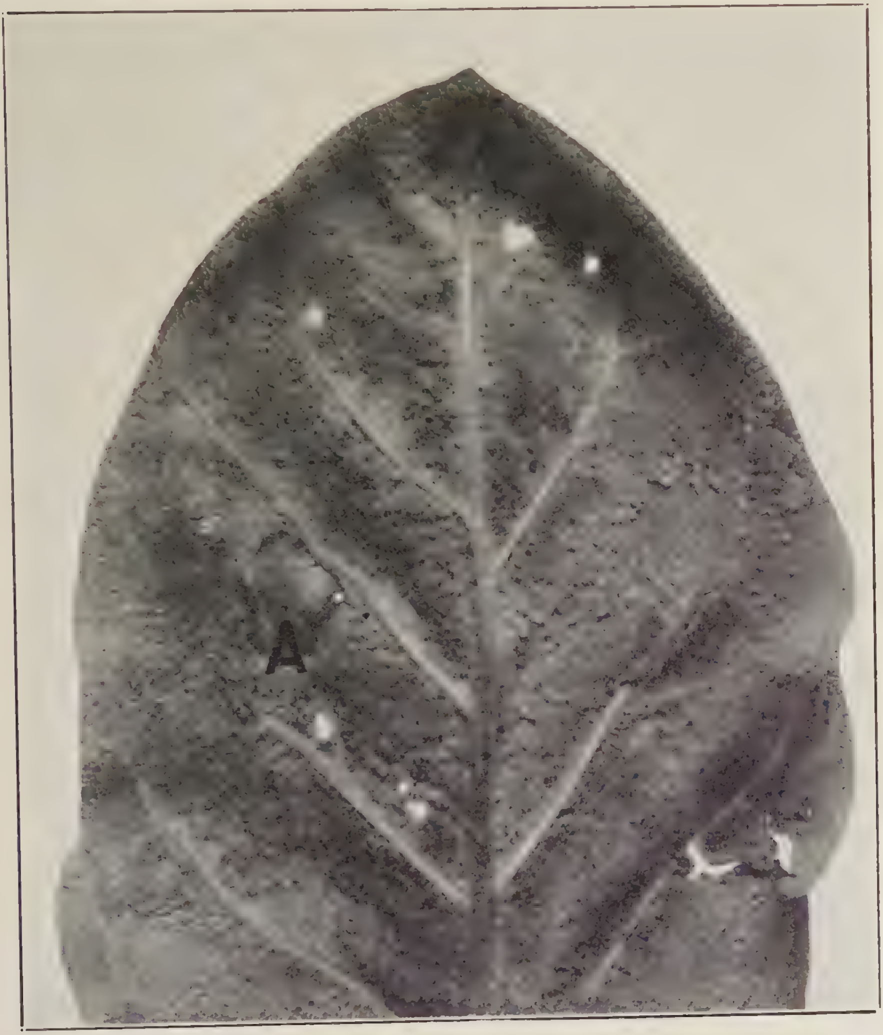

FIG. 1. Fruiting structures of *Peronospora tabacina* on the under side of a leaf of *Nicotiana tabacum*. A large drop of water prevented the formation of fruiting structures at point A.

During the course of rotation many of the sporangiophore branches become entangled with each other and the mature sporangia are dislodged from their attachments as a result of the spring-like action of the branches as they disengage.

The sporangiophores bearing immature sporangia are, however, sensitive to air of varying moisture content and are capable of executing hygroscopic movement. Immature sporangia, however, are firmly attached, since none were observed to be released prematurely by branch entanglement following exposure to currents of dry air.

That the ejection of mature sporangia is not dependent upon entanglement was observed in the case of isolated sporangiophores mounted in a specially devised van Tieghem cell into which air was introduced through side arms at a very low velocity. Air at a constant temperature was bubbled slowly through sulphuric acid-water mixtures and led into the van Tieghem cell. As each bubble was introduced into the saturated atmosphere surrounding the isolated sporangiophores a minute decrease in water-vapor pressure was produced. As the experiment proceeded, the vapor pressure decreased gradually, and the sporangiophore rotated slowly. By alternately increasing the vapor pressure, branchlets bearing sporangia could be observed to rotate to the left or to the right. By slowly decreasing the vapor pressure, a point was reached when abscission occurred between the sterigma and its sporangium. The sporangium seemed to be released forcibly by energy applied at the point of attachment. The stimulus for this energy is attributed to differential stresses set up within the sterigmata.

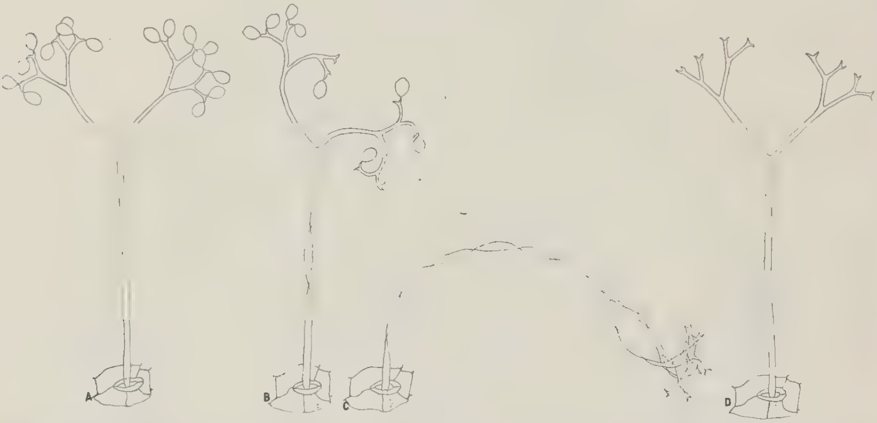

FIG. 2. The cycle of hygroscopic movement executed by a fruiting structure of *Peronospora tabacina*. A. Sporangia mature and attached, no significant vapor-pressure deficit. B. Slight vapor-pressure deficit; sporangial discharge begins. C. Strong vapor-pressure deficit; sporangia completely discharged. D. No signfiicant vapor-pressure deficit; sporangiophore returns to the normal position.

If infected tobacco leaves are kept in a saturated atmosphere sporulation occurs but the sporangia are not released, since no hygroscopic movement takes place. Under such conditions the sporangia merely exhibit proliferation (13). If sporangia, freshly formed on leaves, are held in the saturated atmosphere of a closed container, and if a portion of such leaf is cautiously removed and exposed to "quiet" air during early morning, hygroscopic movements can be observed with a hand lens. The rotation varies from left to right, depending, presumably, upon the vapor pressure of the air striking the sporangiophores. In this connection, it was interesting to note that, although the air may approximate saturation, erratic clockwise and counter-clockwise rotations of sporangiophores were observed. These movements were assumed to be sufficient to cause mechanical entanglement of sporangiophore branchlets and to effect the dislodgment of sporangia. It can be assumed, also, that mature sporangia were ejected by forces applied at their base.

SPORE DISPERSAL OF OTHER MEMBERS OF THE PERONOSPORALES

Other members of the genus *Peronospora* were examined, in the fresh state, to determine if they, too, exhibited hygroscopic mechanisms similar to those described for *Peronospora tabacina.* As a result, *P. parasitica* on *Lepidium virginicum* L., *P. geranii* Peck. on *Geranium carolinianum* L., and *P. halsteadii* Farl. on species of *Ambrosia* were observed to behave similarly.

In studying these downy mildews, it was necessary under certain conditions to mount the leaf bearing freshly formed fructifications under a prefocused microscope and to transport the leaf with its fruiting structures to the microscope, under very humid conditions. Collections were made before sunrise, and the examinations made immediately in a damp, semidark basement. After the material was in place on the microscope stage, a beam of light was focused upon it. After a few seconds, the heat from the light created a local vapor-pressure deficit in the field of vision and violent spore discharge was observed. Merely transferring leaves bearing sporangia from a moist chamber to the microscope stage, under ordinary laboratory conditions, caused mature sporangia to discharge. The delicate nature of the fructifications is correlated with a delicate balance of turgor pressure, which is easily upset by a vapor-pressure deficit when sporangia are mature and ready to abciss. This factor is evidently important in the development of field epidemics.

Dr. Charles J. Nusbaum, in a personal letter to the writer regarding the hygroscopic mechanisms of *Peronospora parasitica* on cabbage (*Brassica oleracea* L.) and *P. effusa* on lambs quarters, (*Chenopodium album* L.), stated, "In each case, the twisting appeared to be in a counter-clockwise direction under the microscope and was very pronounced. . . . The twisting of the main stalk and branches appeared to be nearly simultaneous and quite violent at the start. . . ."

DISCUSSION

The mechanism of sporangial discharge, described for *Peronospora tabacina,* contributes significantly to a better understanding of the life cycle of the parasite in relation to its environment. Numerous field observations over a period of several years have shown that sporangia are formed most abundantly during periods of approximate atmospheric saturation. These periods occur, in the field, during the early hours before sunrise. If saturation persists, the mature sporangia remain attached to the sporangiophore. Rainfall is, therefore, not a significant factor in causing dislodgment, as many have heretofore believed. Dixon and associates (4, page 756) concluded after extensive field observations, that continued rainy weather, combined with long periods of saturation, although conducive to abundant sporulation, inhibits dissemination. Furthermore, Wolf and McLean (13) have shown that under conditions of saturation the mature sporangia merely proliferate *in situ*. These observations, together with those herein reported, seem to warrant the conclusion that downy mildew of tobacco is disseminated *following* atmospheric saturation and *not during* ordinary, continuous rain or during periods with no vapor-pressure deficit in the vicinity of the sporangia.

The period immediately following atmospheric saturation, or near-saturation, seems to be the best for dissemination of and infection by downy mildew fungi. During these periods conditions for sporangial germination are optimum. If sporangia were liberated during rains they would be washed from the air, and wide dissemination would seem impossible.

The combined action of air currents and vapor-pressure deficit cannot be separated successfully under field conditions, although it is possible to do so in the laboratory. Even though 100 per cent relative humidity prevails in the field, a slight rise in local air temperatures with the oncoming day will stimulate both sporangial discharge and air movement.

The mechanism of sporangial discharge seems to result from two nearly inseparable factors: mechanical entanglement of the sporangiophore branches, and the ejection of sporangia from their sterigmata by energy applied at the point of sporangial attachment. The exact nature of this latter process is yet to be determined; but, since the entire fruiting structure of *Peronospora tabacina* is developed in a few hours, and since its formation is dependent upon the presence of a nearly saturated surrounding atmosphere, it seems highly probable that the structure of the cell wall is not only very delicate, but also very sensitive to water vapor. Longitudinal thickening of the sporangiophore wall could account for differential stress during desiccation.

The fact that all sporangiophores rotated in unison, and in the same direction, suggests that, whatever the nature of the cell wall may be, it is probably heritable and a normal character of many species of *Peronospora*.

Weston (10) discusses the early literature dealing with the rate of fall of various air-borne spores and concludes that only a very slight air movement is sufficient to carry the Philippine Sclerosporas of maize significant distances. Wolf and associates (12) were able to trap from the air the sporangia of several downy mildews, including 4 of the 5 species discussed in this report. Newhall (8) was able to trap the spores of *Peronospora destructor* (Berk.) Caspary from the air over onion fields. Leach (7) and Doran (5) have, also, reported evidence that downy-mildew spores are wind-borne.

From the foregoing evidence it seems highly probable that the hygroscopic mechanism of *Peronospora tabacina*, *P. parasitica*, *P. geranii*, *P.*

halsteadii, and *P. effusa* has a definite function in the life history of these organisms. It also seems probable that other members of the genus have a similar mechanism for sporangial liberation. An understanding of these facts will undoubtedly lead to a better appreciation of the mode of spore dissemination and of disease development among the downy mildews.

SUMMARY

Sporangial dispersal, caused by changes in the moisture content of the air has been described for *Peronospora tabacina*, *P. parasitica*, *P. geranii*, *P. halsteadii*, and *P. effusa*.

Sporangial dispersal in these members of the Peronosporales begins with incipient drying, and is concluded by hygroscopic distortion of the aerial fructifications.

The mechanical action of wind and rain, during periods of atmospheric saturation, is not believed to be responsible for the dispersal of sporangia of these species of *Peronospora*.

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COLD INJURY OF FLUE-CURED TOBACCO SEEDLINGS[1]

J. A. PINCKARD AND LUBEN SPASOFF BOZOVAISKY[2]

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INTRODUCTION

A disorder of young tobacco seedlings, known locally as "white bud" or "yellow bud," has been observed in the bright-tobacco area of Virginia for the past several years. The disease has been noted only in the early spring about the time seedlings have a spread of ¼–2 inches.

The first symptom of this disorder (Figs. 1 and 2) is xanthosis of the young leaves enclosing the growing point. The result is a conspicuous and characteristic white or yellowish-white apical bud. As the small bud leaves expand, yellowing becomes evident along the margins but may disappear as the leaves grow older and no other noticeable symptoms develop. In more severe forms of the disorder necrosis of the marginal cells is a result. In such cases the young leaves may become permanently or temporarily distorted. If this happens, the plants usually suffer temporary retardation of growth. Thickened, glazed leaves of occasional seedlings usually are present in seed beds wherein this injury is manifest.

The cause of "white bud" in tobacco seedlings has been variously diagnosed as either too much or too little of one or another constituent of the seed-bed fertilizer. It also has been assumed to be an expression of frenching or of one of the numerous viruses attacking tobacco. Valleau and Johnson[3] have encountered this disease in Kentucky, and have correctly diagnosed it merely as an expression of cold injury.

The purpose of this paper is to describe experiments leading to the artificial reproduction of the above described cold injury of young tobacco seedlings.

MATERIALS AND METHODS

Seedlings of both the burley and flue-cured types of tobacco were grown in flats of a rich, loamy soil under strong illumination to encourage rapid, vigorous growth. When the plants were approximately 1½ to 2 inches across, they were subjected to night temperatures of approximately 40° F., for several nights, for preliminary hardening. The night temperatures were then further reduced to 33° F., and below, for stated periods of time to study the symptoms induced by low temperatures upon the seedlings. Refrigerated plant-growing chambers, described elsewhere,[4] were employed for this purpose.

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[1] Published with the approval of the Director of the Virginia Agricultural Experiment Station.

[2] Formerly Associate Plant Pathologist, and Senior Agricultural Scientific Aide, respectively, Virginia Agricultural Experiment Station. The writers also wish to express their gratitude to F. A. Wolf and W. A. Whitney for assisting in many ways.

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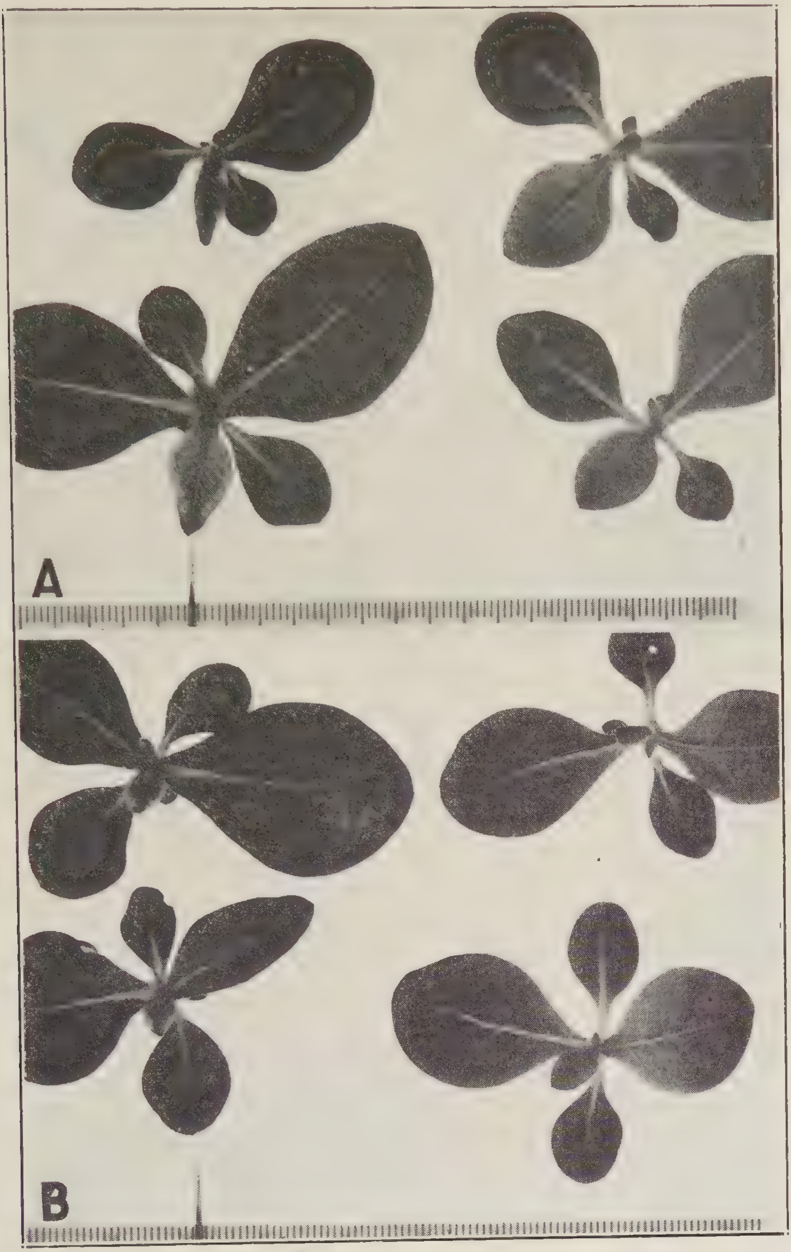

FIG. 1. Cold injury ("white bud") of flue-cured tobacco seedlings. A. Left, field-grown plants unprotected from cold during the night; right, plants protected by heavy muslin fumigation covers. B. Left, artificially-induced cold injury; right, untreated seedlings. Photographed approximately natural size, 5 days after exposure to cold. Contrast filter employed.

After subjecting the seedlings to various cold treatments they were again placed in a cool greenhouse, in strong illumination, and the effect of the cold treatment noted.

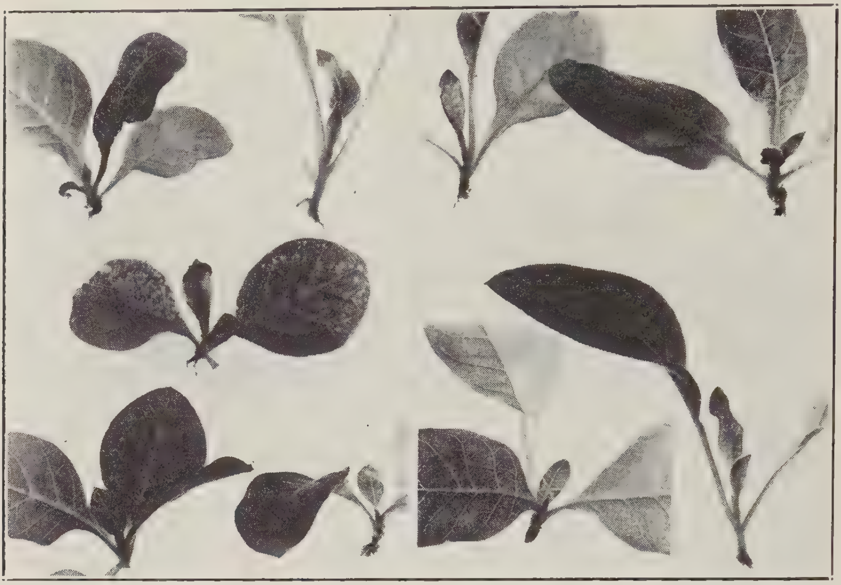

FIG. 2. Artificially-induced cold injury of burley tobacco seedlings brought about by air temperatures of 29 to 32° F. for a period of 16 hours. Photographed approximately 9 days after exposure.

RESULTS

Air temperatures between 31 and 33° F. for 15 hours sufficed to freeze both the soil and the leaves of seedlings growing in small flats. After the plants had thawed, wilting was observed; but the plants recovered without damage and were indistinguishable from untreated check plants.

Air temperatures between 29 and 32° F. for 16 hours were sufficient to cause the general cold-injury symptoms. Figure 2 shows these plants 9 days after treatment. Some plants in this series of experiments were not visibly affected by cold treatment; others exhibited various types and degrees of injury. If damage occurred, however, the young leaves enclosing the bud were always affected, and yellowing developed within a few days.

Air temperatures between 27 and 29° F. for 3 hours destroyed many of the buds outright, leaving the larger leaves unaffected and attached to a dead central axis. Several plants in this series of experiments showed glazed, thickened leaves, and the characteristic yellow apical bud leaves.

Air temperatures between 24 and 26° F. for $1\frac{1}{2}$ to $2\frac{3}{4}$ hours resulted in typical "white bud" symptoms (Fig. 1, B), similar to those of field-grown plants (Fig. 1, A). Thickened leaves with glazed surfaces appeared more abundantly at these temperatures than in any of the others. Both types of cold injury were quite apparent on the 5th day following cold treatment.

Field occurrence of the "white bud" type of cold injury was never observed in tobacco seed beds covered during cold nights with heavy muslin covers of the type used for fumigation and control of tobacco downy mildew. It did occur, however, following cold nights, in adjacent uncovered check seed beds. Figure 1, A, illustrates field-grown plants from adjacent uncovered and covered seed beds, while figure 1, B, shows a similar condition artificially induced.

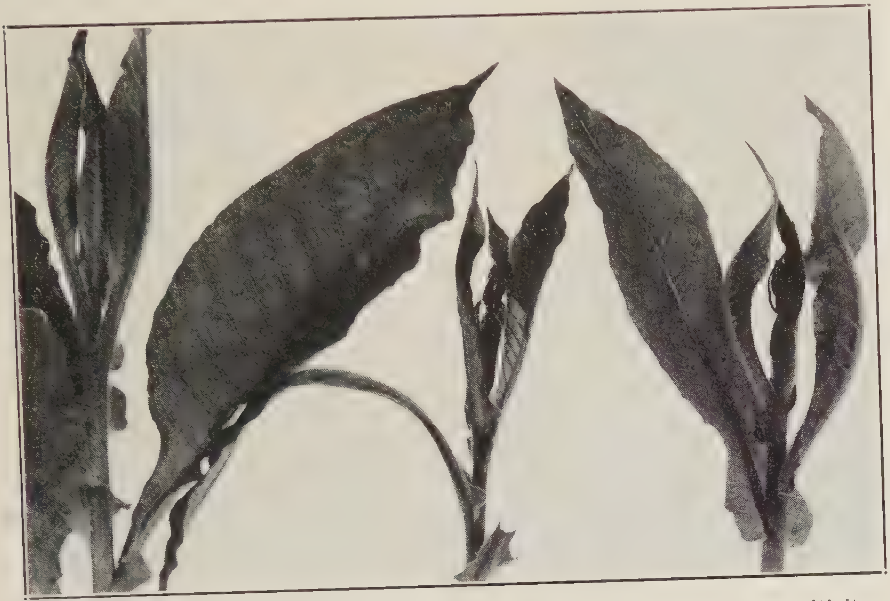

FIG. 3. Natural frost injury of old tobacco plants showing typical sensitivity of immature tissues. Reduced.

Cold injury, as a result of frost, on old, field-grown plants (Figs. 3 and 4) appeared to be similar in many respects to cold injury to seedlings. The young marginal leaf tissue and the growing point, as in the case of seedlings, were most susceptible to cold, while peculiar patterns and blanching developed on old leaves.

DISCUSSION

Tobacco seed beds in Virginia usually are prepared and sown during the first period of warm weather in January. The seeds lie dormant in or on the ground until the warm weather of spring brings about germination and subsequent growth. Late snowfall or freezing weather may follow warm periods after plants have emerged and relatively large leaves have developed. Depending upon local conditions, ice may form on the leaves without causing significant damage.

Experiments reported in this paper indicate that both the leaves of prehardened tobacco seedlings and the soil supporting them may become solid from ice crystals for several hours, without causing significant damage to the plants. It appears, however, that if temperatures drop below 30 or 31°

F. for a few hours damage may result. As the temperature decreases, as may be expected, the injury increases. The first symptoms of injury appear to be a blanching of the primordial tissues, which results in the symptoms commonly observed as "white bud." Plants showing such symptoms may have been exposed to temperatures as low as 24 to 26° F. but for not more than 2 or 3 hours.

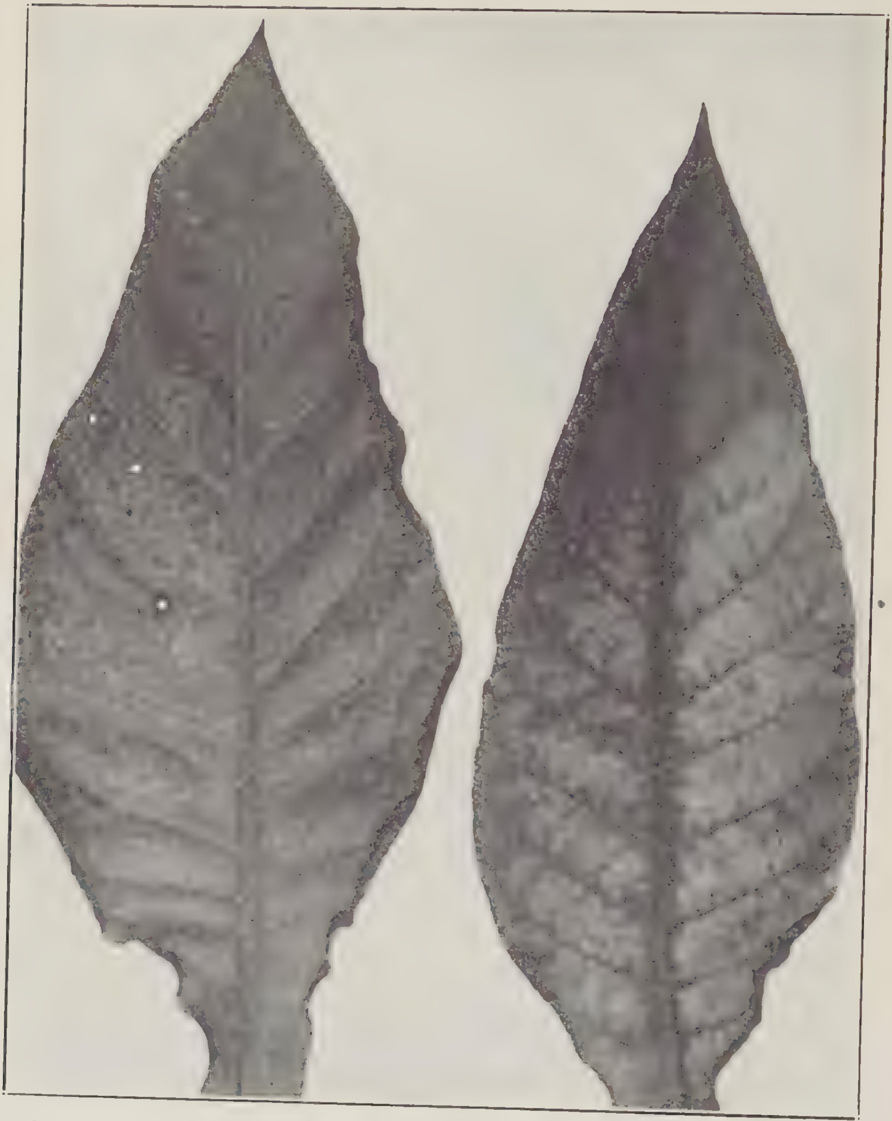

FIG. 4. Natural frost injury of old tobacco leaves showing peculiar patterns and blanching. Reduced.

As cold injury became more severe, the marginal tissues of very young leaves were destroyed progressively toward the base. The central axis of the plant and the growing point were destroyed before older leaf tissue was harmed. In older plants similar conditions were encountered, except that on old leaves irregular color patterns and blanching appeared.

SUMMARY

Incipient cold injury to tobacco seedlings, of both the burley and flue-cured types, resulted in bleaching of the bud leaves. This disorder, common in early spring, appears to be induced by an exposure of 2 or 3 hours at temperatures between 24 and 26° F., or in 15 to 16 hours at temperatures between 29 and 32° F. Typical symptoms of the so-called "white bud" disease, a phase of cold injury, appeared 4 or 5 days after plants were exposed to these temperatures.

The first tissues in tobacco leaves to succumb to cold were those at the margins of very young leaves. This appeared to be true of both seedlings and older plants. More intense cold destroyed the apical meristem in both young and old plants, while cold injury to mature leaves resulted in irregular patterns and blanching.

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A SEED–BORNE MOSAIC OF ASPARAGUS BEAN, VIGNA SESQUIPEDALIS

W. C. Snyder

(Accepted for publication August 27, 1941)

During a period of 1938 when frequent greenhouse plantings of asparagus bean, *Vigna sesquipedalis* Wight, were being made, it was observed that a small percentage of the plants developed mosaic symptoms. The seed involved was from a commercial lot purchased under the name of Yardlong Bean. This paper is concerned with the description of this disease and the determination of its nature.

SYMPTOMS

The symptoms of asparagus bean mosaic (Figs. 1, 2) are marked by a light and dark green mosaic, frequently attended by a downward rolling of the leaflets. Leaf rolling is often pronounced. The dark-green areas tend to become puffy and result in mild rugose symptoms, or leaf distortion (Fig. 2, C and D). Variations in the mosaic pattern occur, as they do in the case of common bean (*Phaseolus vulgaris* L.) mosaic caused by *Bean virus 1* Pierce. Frequently the dark-green areas form broad bands along the main veins of the leaflet, as seen in figure 1, the remainder of the leaf being lighter green. Occasionally the primary leaves of plants infected through seed transmission of the virus show mild mosaic symptoms or slight leaf distortion and stunting (Fig. 2, A). Infected plants often are dwarfed, especially if infection has taken place through the seed, and growth may be less robust than in normal plants. Severely affected plants frequently set little or no seed.

JUICE TRANSMISSION

Juice expressed from diseased plants was used to inoculate healthy plants obtained from a stock of disease-free seed, employing the rubbing method of inoculation aided by the use of carborundum as an abrasive. The infection of over a hundred healthy plants was obtained at the outset of the work in this way with the production of the characteristic mosaic symptoms shown in figures 1 and 2. Plants held as controls remained different tests, ranging from less than 10 per cent to more than 90 per cent. healthy.

Usually 9 to 28 days elapsed from time of inoculation until appearance of symptoms, apparently depending upon conditions prevailing in the greenhouse. The percentage of infection obtained varied considerably in the

APHID TRANSMISSION

The pea aphid, *Macrosiphum pisi* (Kalt.), was used to test the insect transmissibility of the virus. Noninfected aphids were reared on plants of *Vicia faba* L. grown under cages. Colonies were transferred to mosaic-infected plants of asparagus bean, where they were allowed to feed 2 days before being transferred to healthy plants in numbers of 10 to 25 aphids per plant. After 2 days the aphids were removed by thoroughly spraying the plants with nicotine sulphate solution. Controls consisted of healthy asparagus bean plants on which like numbers of noninfective aphids were placed. Of 14 plants on which infective aphids had been allowed to feed, 10 became infected with mosaic. No mosaic developed in 9 plants on which noninfective aphids were fed. These results sufficed to demonstrate that the virus belongs to the group of aphid-transmitted viruses, and no further tests with aphids were made. It seems probable that other species of aphids which feed naturally on asparagus bean also may be vectors of the virus,

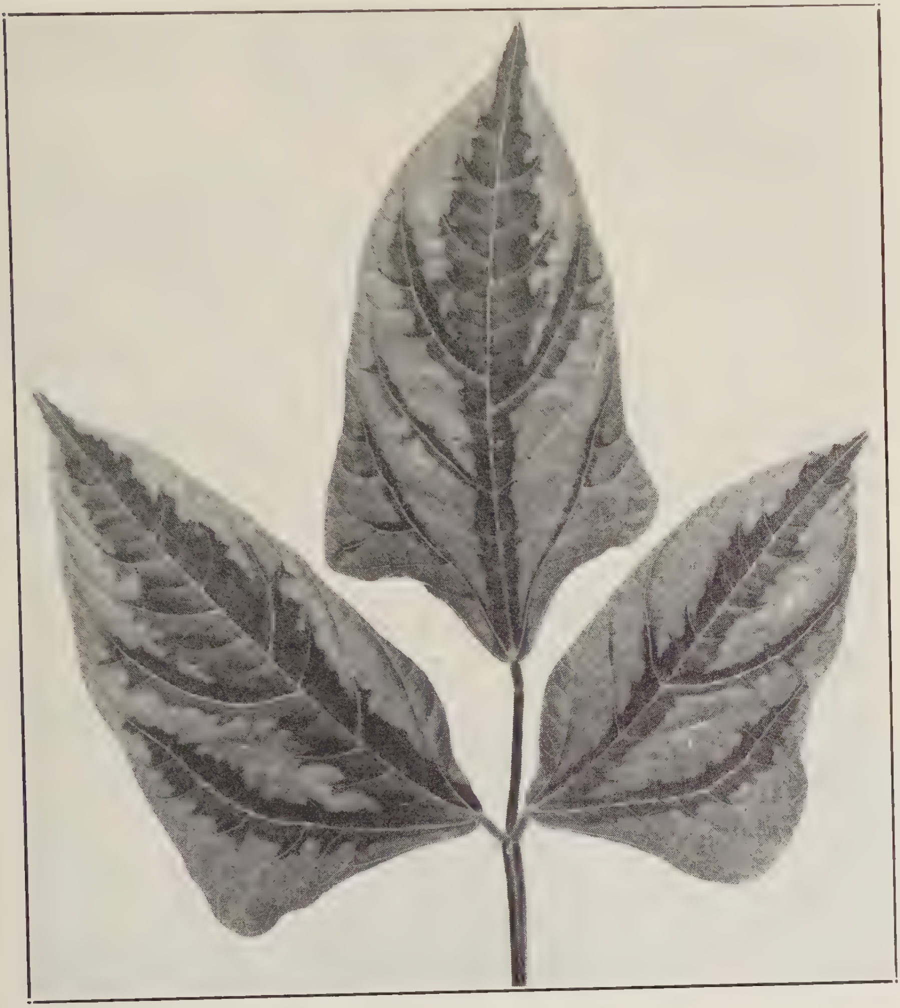

FIG. 1. Vein-banding symptoms of asparagus bean mosaic.

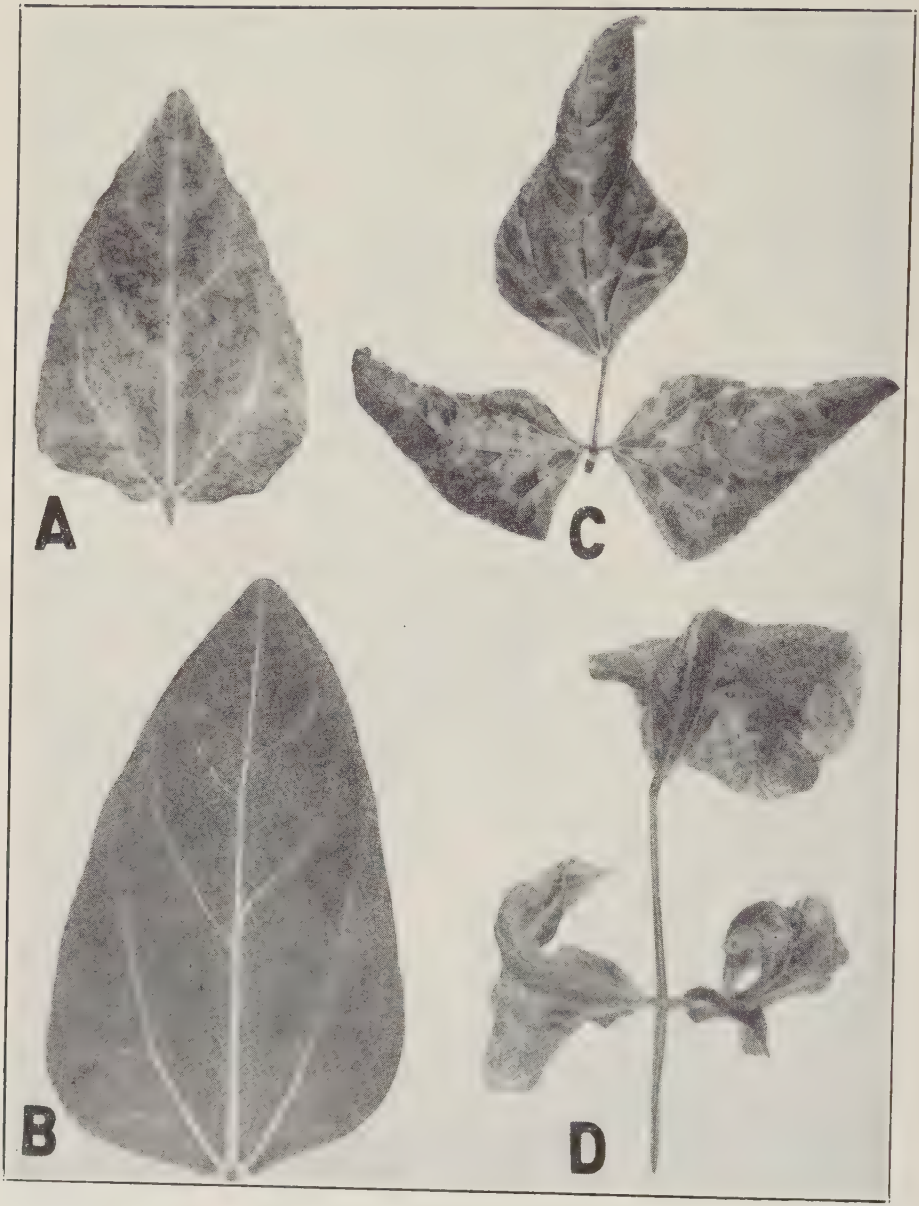

FIG. 2. A, first symptoms of asparagus bean mosaic on a primary leaf infected through the seed; B, healthy primary leaf of the same age as in A; C, rugose mosaic, and D, severe leaf distortion symptoms of asparagus bean mosaic on trifoliate leaves.

judging from the experience gained from studies of other aphid-transmitted legume viruses.

SEED TRANSMISSION

It has already been mentioned that the appearance of this mosaic of asparagus bean was first seen in plantings from a certain lot of commercial seed grown in a greenhouse. In a count made of infected and healthy plants from a planting of this seed lot, 4, or 3.3 per cent, were infected out of a total of 121 plants.

A few infected plants were allowed to mature in the greenhouse, and the seed from them replanted. Of 109 plants grown from the seed set by diseased plants, 41, or about 37 per cent, developed mosaic symptoms. These figures illustrate a seed transmissibility of the virus resembling that of the well-known common bean-mosaic virus. The seed-borne nature of the virus serves to distinguish it from most other viruses, as well as to stress its similarity to those of common bean mosaic, cowpea (*Vigna sinensis* Endl.) mosaic, and soybean (*Soja max* Piper) mosaic (Soybean virus 1 Pierce).

HOST RANGE

Attempts to infect other plants, particularly legumes, have mostly failed. Infection of cowpea has been obtained, with symptoms resembling those of cowpea mosaic, but only in certain instances has the infection of common bean (*Phaseolus vulgaris*) been successful. In the latter case symptoms have not agreed with those of common bean mosaic. Plants inoculated, but that so far have not shown infection, include catjang (*V. catjang* Walp.), Midwest soybean (*Soja max*), Perfection and Laxtons Progress pea (*Pisum sativum* L.), common and Peruvian alfalfa (*Medicago sativa* L.), *Acer arietinum* L., *Lathyrus odoratus* L., *Melilotus alba* Desr., *M. indica* All., *Trifolium repens* L., *T. pratense* L., *Nicotiana tabacum* L., and *N. glutinosa* L.

Likewise, an attempt to infect the asparagus bean with the common bean-mosaic virus was without results. It would appear from these tests on host range, although not exhaustive, that the virus causing this mosaic of asparagus bean is narrowly limited in its infectivity, and that it is probably largely confined in nature to the genus *Vigna*.

PROPERTIES OF THE VIRUS

The thermal inactivation point, dilution end point, and longevity of the virus were determined in the usual way. The expressed juice from infected plants was strained through cheesecloth, treated, and then inoculated into healthy asparagus beans by the rubbing method. The results obtained are shown in table 1.

TABLE 1.—*Physical properties of the asparagus bean-mosaic virus*

Thermal inactivation			Dilution tolerance			Longevity *in vitro*		
Temp.	No. plants inoculated	No. plants infected	Dilution	No. plants inoculated	No. plants infected	Days	No. plants inoculated	No. plants infected
Room	35	17	None	33	21	None	34	21
55° C.	37	15	1/1000	30	2	1	41	9
60° C.	34	0	1/3000	27	0	2	24	1
65° C.	32	0	1/6000	21	0	4	23	0

These results (Table 1) show that the virus in juice extracted from diseased plants was inactivated by heating it for 10 minutes at a temperature of 60° C.; by diluting it to a concentration of 1–3000; and by keeping the juice in stoppered test tubes for about 2 days at a constant temperature of 21° C.

DISCUSSION

Mosaic diseases of asparagus bean, cowpea, and catjang were observed in field plots in Indiana by Gardner (1) in 1925. No attempt was made to study the viruses causing these diseases other than to demonstrate that the cowpea mosaic was seed-borne (2, 3).

Nelson (4) cites the asparagus bean as a host of common bean mosaic virus, but others, including Pierce (5), and Zaumeyer and Wade (6), were unable to confirm Nelson's findings. The recent studies of McLean (8) indicate that the cowpea-mosaic virus may cause a mosaic of catjang, but, apparently, the asparagus bean was not tested. That the asparagus bean virus may differ from the cowpea virus is suggested in the present paper by the failure of the former to infect catjang, and by the difference between the thermal inactivation points of the two viruses (McLean reports 72° to 75° C. for the cowpea virus). In addition, the symptoms produced on cowpea have not always been the same as those described for cowpea mosaic. These distinctions are not large, and they suggest that closely related viruses or perhaps even strains of one virus may be involved in the two diseases.

On the bases of symptoms, seed carriage, aphid transmissibility, and properties, the asparagus bean mosaic clearly belongs to the group of viruses that cause, respectively, the seed-borne mosaics of common bean (9), soybean (7) and cowpea (3). Each of these viruses appears to be restricted in nature to one or to a few species, and this also appears to be true in the case of the asparagus-bean virus. Here is an instance where several apparently distinct virus diseases show a remarkably close relationship in respect to cause. In a biologic sense these viruses might well be considered to be physiologic forms of the same virus species. They show similar thermal inactivation points (McLean's results on the cowpea virus place it somewhat out of line with the other viruses in respect to the thermal inactivation point), longevities *in vitro*, dilution tolerance, seed transmissibility, aphid and mechanical transmissibility, and limited host ranges, as well as a similarity in symptoms. Essentially then, these viruses differ primarily in their specialization as to host, and might be considered as variants of a common virus, which have become more or less adapted to specific hosts.

SUMMARY

A mosaic disease of asparagus bean, *Vigna sesquipedalis*, recovered from a lot of commercial seed, is described.

The causal virus was transmitted from diseased to healthy plants both by the mechanical inoculation of expressed juice and by the pea aphid.

The virus is seed-borne, and in this respect it closely resembles the viruses that cause common bean mosaic, cowpea mosaic, and soybean mosaic.

Properties of the virus are shown to be as follows: thermal inactivation point, between 55° and 60° C.; longevity *in vitro*, about 2 days; and dilution end point, nearer 1/1000 than 1/3000.

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INJURY TO TOMATO SEED IN DISINFECTION

J. H. Miller and Raymond Grogan

(Accepted for publication September 6, 1941)

While making routine tests for germination and contamination of treated tomato seed, the senior writer has frequently received preliminary samples from commercial houses that showed a very marked loss of viability. Then, later examinations of samples from the entire lot treated in bulk often failed to show the low germination of the first samples. The method of treating was the same in both cases, so it seemed possible that there might be an important factor in the ratio between the quantity of seed and the volume of solution.

The object of this investigation then was to determine the effect on germination of varying the proportion of seed to solution. The relation of this varying ratio to the residual mercury content of the solution will be considered in a later paper.

MATERIALS AND METHODS

This experiment was divided into the following parts:

1. The quantity of seed was constant at 10 g., and the volume of solution was varied from 10 cc. to that amount of seed, to 100 cc. with 10-cc. intervals, and then continued up to 500 cc. with 100-cc. intervals. Germination tests were run after each trial.

2. The volume of solution was constant at 100 cc., and the amount of seed was varied from 1.5 g. up to 48 g. The intervals were secured by doubling the quantity of seed each time. Enough classes were placed in this experiment to check the high points of the first. This test reverses the first in that it begins with a wide ratio and ends with a narrow one. Germination tests followed as above.

3. Disinfection tests were made from each trial by plating out 200 seeds on dextrose-potato agar in Petri dishes and counting the number showing contaminations on the fourth day. This was for the purpose of determining if there is a relationship between the effectiveness of treatment and the varying seed-solution ratios.

The seed used in this investigation was Stark's Earliana variety from the 1940 crop. These were nontreated, and handled as ordinary commercial seed.

Two standard solutions were used for treating.

1. Mercuric chloride, C.P., at rate of 1 g. to 3000 cc. of water. Seeds were stirred constantly while in the solution, and aften ten minutes they were taken out and rinsed in three changes of distilled water, and then dried on paper towels.

2. Ethyl mercury phosphate, 5 per cent (New Improved Ceresan), at rate of 1 g. to 1200 cc. of water. These were treated as above for 10 min.,

but were dried without rinsing. The concentrations remained constant in all trials.

The germination tests were made in a standard hot-water-jacket germinator. Two hundred tomato seeds from each different solution were placed on blotters and counts were made at 4, 7, and 11 days, respectively.

The entire experiment was executed 3 times, and the curves shown in the accompanying graphs represent the average of the three.

EXPERIMENTAL RESULTS

There were no material changes in the percentage germination at the end of the 4th day (Fig. 1) until a ratio of 1 part of seed to 6 of solution had been reached for the Ceresan and 1 to 5 for the mercuric chloride. After this both rather consistently caused a decreased germination, and at 1 to 50 only 1.15 per cent germinated for Ceresan and 0.83 per cent for mercuric chloride. The seed treated in the latter solution showed a lower germination in all solutions, except the 1 to 7 and 1 to 9, when the two were about equal.

The curve of the 7th day (Fig. 1) also shows a decreased germination with the widening ratios after 1 to 6 for Ceresan and 1 to 8 for mercuric chloride. The 1 to 50 ratio caused a decline to 7.98 per cent for Ceresan and 2.33 per cent for mercuric chloride.

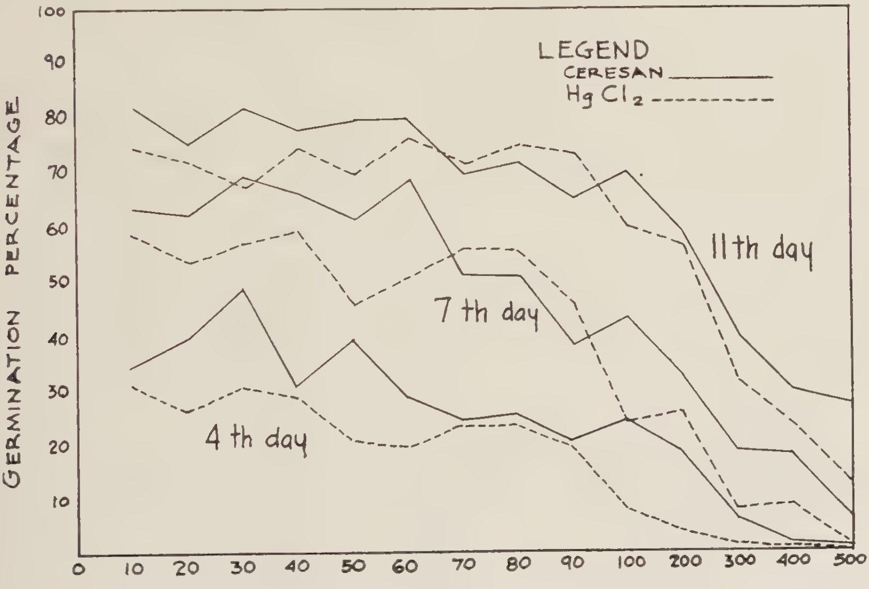

FIG. 1. Curves showing the effect on germination of varying the ratio between the volume of treating solution and the quantity of seed.

The final germination counts were made on the 11th day and then (Fig. 1), there was a significant decline in the gradient following a solution of 1 to 6 for Ceresan and 1 to 9 for mercuric chloride. When 1 part of seed was placed in 50 cc. of solution the final germination was 27.74 per cent for Ceresan and 13.0 per cent for mercuric chloride.

In the second experiment, where the volume of solution was maintained a constant and the quantity of seed was varied the curves (Fig. 2) are reversed. The wide ratio of 1.5 parts of seed to 100 cc. of solution resulted in 1.3 per cent for the 4th day, 3.9 per cent for the 7th and 17.1 per cent for the 10th day for Ceresan, and 2.75 per cent, 17.17 per cent, and 34.3 per cent, respectively, for mercuric chloride. On the other hand, the narrow ratio of 48 to 100 produced a germination of 25.8 per cent, 53.8 per cent and 72 per cent for the former treatment and 25.1 per cent, 61.9 per cent, and 78 per cent for the latter.

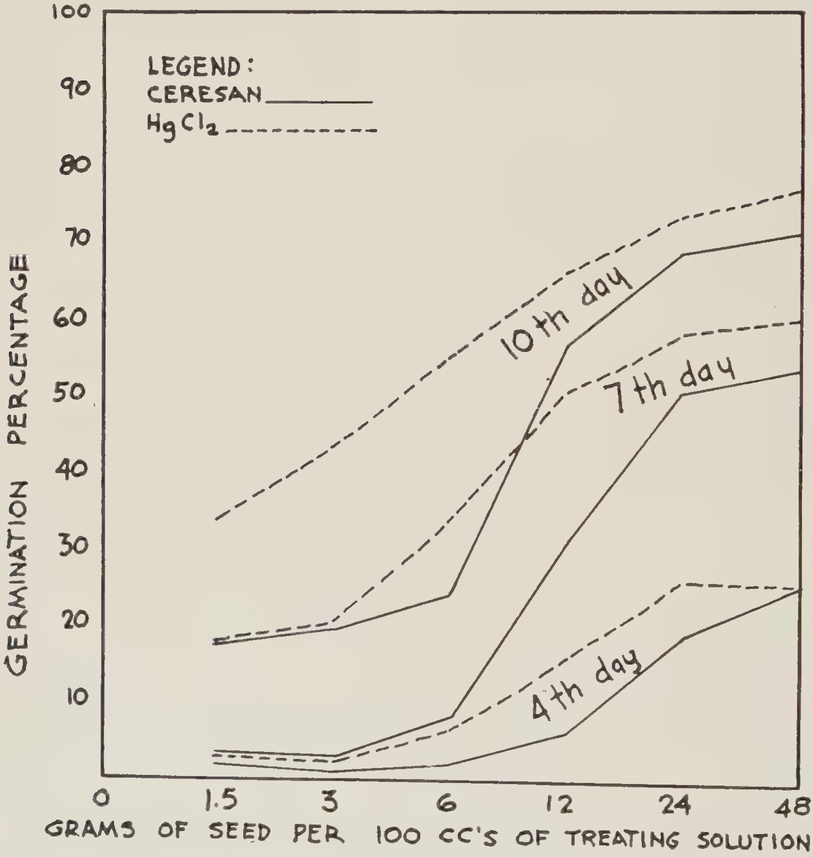

FIG. 2. Curves also showing the effect on germination of different seed-solution ratios with the variable being the quantity of seed.

Both experiments show the same material decrease in seed germination following a widening of the ratio, with a critical point at about 1 of seed to 7 to 9 parts of solution.

CONTAMINATION STUDIES

In seed treatment the two chief points to be considered are effectiveness and seed injury. The following table gives the results of plating out 200 seeds from each treating trial.

TABLE 1.—*The effect on seed contamination of varying the volume of solution*

Seed	Solution	Ceresan contamination	Mercuric chloride contamination
g.	*cc.*	*Per cent*	*Per cent*
10	10	41.8	20.0
10	20	5.8	1.8
10	30	3.3	0.0
10	40	1.8	0.25
10	50	0.5	1.1
10	60	0.0	0.0
10	70	0.0	0.0
10	80	0.0	0.0
10	90	0.0	0.0
10	100	0.0	0.0

No organisms grew from the seed after treatment of 1 to 6.

TABLE 2.—*The effect on contamination of varying the quantity of seed*

Seed	Solution	Ceresan contamination	Mercuric chloride contamination
g.	*cc.*	*Per cent*	*Per cent*
1.5	100	0.0	0.0
3.0	100	0.0	0.0
6.0	100	0.0	0.0
12.0	100	0.5	6.0
24.0	100	0.0	9.0
48.0	100	3.0	12.5

This second table was obtained by plating out seeds from the germination trials of the second experiment. This shows that no organisms appeared beyond a proportion of 12 to 100, or 1 to 8½. The wider ratios produced complete disinfection of the seed.

The Ceresan solutions in the contamination studies (Table 1) did not produce as efficient disinfection as mercuric chloride up to 1 to 4 ratio, and in the corresponding germination graph (Fig. 1) the reduction in germination is also not so great. Then in table 2 the reverse is shown; that is, the Ceresan is ahead of the mercuric in disinfection and the graph (Fig. 2) shows a greater decrease in germination. It is, therefore, evident from this study that as the treating solution becomes more efficient there is a resulting sacrifice of seed viability. This, however, is not a general rule, as past laboratory experience has shown that heavily infected seed often germinates better after treatment.

SUMMARY

The experimental evidence indicates that when the ratio between quantity of seed and volume of treating solution is increased above 1 to 8 the germination of tomato seed is progressively impaired.

The curves for Ceresan and mercuric chloride are not significantly separated in figure 1, and in figure 2 the trends are reversed. So one cannot logically predict a difference in germination depression between the two from the results of this investigation. Both of them, however, show the same downward trend of the curves in wide ratios.

The ratio of 1 to 8 produced complete disinfection and did not cause a too serious drop in germination.

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GROWING NEW ROOT SYSTEMS BY SOIL BANKING—A PROMISING METHOD OF REJUVENATING TREES ATTACKED BY ROOT DISEASES

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Although the life histories of many of the organisms causing root rot of woody plants have long been understood, little progress has been made in the control of these diseases. As a whole, this group of diseases has received much less attention by plant pathologists than most other groups, and certainly far less than their economic importance merits. In the case of the mushroom or toadstool root-rot fungi, it is universally conceded that it is difficult to adopt effective control measures and that if trees become attacked their treatment is tedious and expensive and the results uncertain. However, varying degrees of success have been reported by a number of investigators.

The methods that have been used most widely thus far in the treatment of root diseases of woody plants have been confined largely to (a) surgical treatment, involving excision of all diseased tissues and dead roots, followed by painting the exposed wood surfaces with various paints, or even all the exposed roots with various disinfectants; (b) the aeration method in which the soil is removed from under the base of the attacked tree and the adjacent main roots to check the development of the causal organism by desiccation; (c) application to the soil of various chemicals in an effort to arrest the development of the causal organism or eradicate it, preferably without sacrificing the attacked tree; and (d) the isolation method, accomplished by digging of trenches or erection of barriers around infected areas to prevent further spread of the fungus through the soil. Various combinations of these methods have, of course, been employed.

The first two of the above methods have proved fairly effective, especially when combined, if applied before a large percentage of the roots are killed. The third has proved ineffective so far as saving attacked trees is concerned, though, if one is prepared to sacrifice the attacked trees and sometimes also adjacent unattacked ones, injection of carbon bisulphide in a network of holes in the soil has proved an effective means of eradication, at least in light, porous, fairly dry soils and under favorable temperature conditions. Even with this method, because of difficulty of eradicating the mycelium within the interior of large roots, it is recommended that such roots be pulled or dug out before treating the soil. The last method, which was suggested for combatting the spread of *Armillaria mellea* before it was found possible to eradicate the fungus by soil disinfection, appears impractical.

This paper is designed to direct attention to the value of another promising method that appears to be not widely known or appreciated, though it has been used in Florida for several years. It consists in banking soil around the bases of attacked trees to stimulate development of new roots. The readiness with which citrus trees and many other trees and shrubs develop adventitious roots when the soil is worked up above the normal ground level has suggested the possibility of utilizing this natural tendency to develop adventitious roots as a means of growing new root systems on trees whose life is threatened by various root diseases. Instances have been observed frequently where citrus trees, killed back in the freezes of 1894–5, have developed roots in the interiors of the hollow shells left by the rotting away of the central wood of the original trunks, in the litter accumulated therein. Indications of new root development are seen frequently also on the exterior of citrus trees, where a callus has formed following some basal injury and the soil has been worked up somewhat above the usual level. Several instances have been noted in Florida citrus groves where the soil was allowed to remain in place for a few years after banking for cold protection during the winter season. In such instances, when the banks were torn down subsequently, roots as large as a man's arm were found to have developed, giving the trees a secondary system of brace-roots similar to a red mangrove (*Rhizophora mangle*).

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In 1924, and subsequently, Jeffries (3, 4, 5) reported a method of giving citrus trees new root systems through partial ringing, followed by banking. This method was discovered following the usual practice of banking, in the fall to guard against freezing, some young trees, which had been partly girdled to force bud growth. When the banks were removed at the end of the winter season a splendid new root system was found to have developed from the margin of the callus formed immediately above the girdle. He found this method desirable for giving trees a new root system when budded to an undesirable rootstock, and preferable to inarching. He also suggested the applicability of this method for saving sweet seedling orange trees attacked by foot rot.

In 1926, Höstermann (1) reported that *Caragana arborescens* and *Laburnum vulgare* could be propagated successfully by ringing followed by mounding with soil. Shoots of both plants ringed in August developed extensive root systems by November from the callus formed above the wounds. He later (2) extended these experiments to 43 species of fruit stocks, fruit varieties, nuts, ornamentals, etc. Compared with ringing, a tight wire band gave more favorable results with respect to callus formation and rooting, making possible the vegetative propagation of 31 of the 43 species.

In 1926, the writer (6) briefly reported the successful rejuvenation of old citrus trees, nearly dead from basal girdling by foot rot (*Phytophthora parasitica*), which had been accomplished successfully by a grower at Umatilla, Florida, by the simple expedient of merely banking soil around the bases of the trees without any preliminary treatment. This experiment was begun on a number of old, half-dead seedling orange and grapefruit trees in 1920, and was subsequently extended to other trees after its success became apparent. In the initial trees treated, which were first examined in 1925, a rough framework was built about the base of each tree treated and this was filled with two wagon-loads of clay, covering the base of the trunk to a height of from 15–18 inches. The framework was used to better retain the soil about the bases. Sand has been used for this purpose in various parts of the State, but is much more subject to washing away when no framework is provided and is less retentive of moisture. In from 2–5 years a more or less profuse growth of new roots developed from the margin of the callus formed above the bases of the partly girdled trunks. In those cases where the soil was washed away for examination about 2 years after banking, good-sized roots were found bridging basal lesions where the wood had become extensively decayed (Fig. 1). In all cases the trees no longer exhibited the thin, yellow tops characterizing trees attacked by foot rot, but, following the establishment of the new root systems (Fig. 2), had developed healthy tops with good fruit production. The trees thus treated in this grove still continue to thrive. This simple treatment has added many years

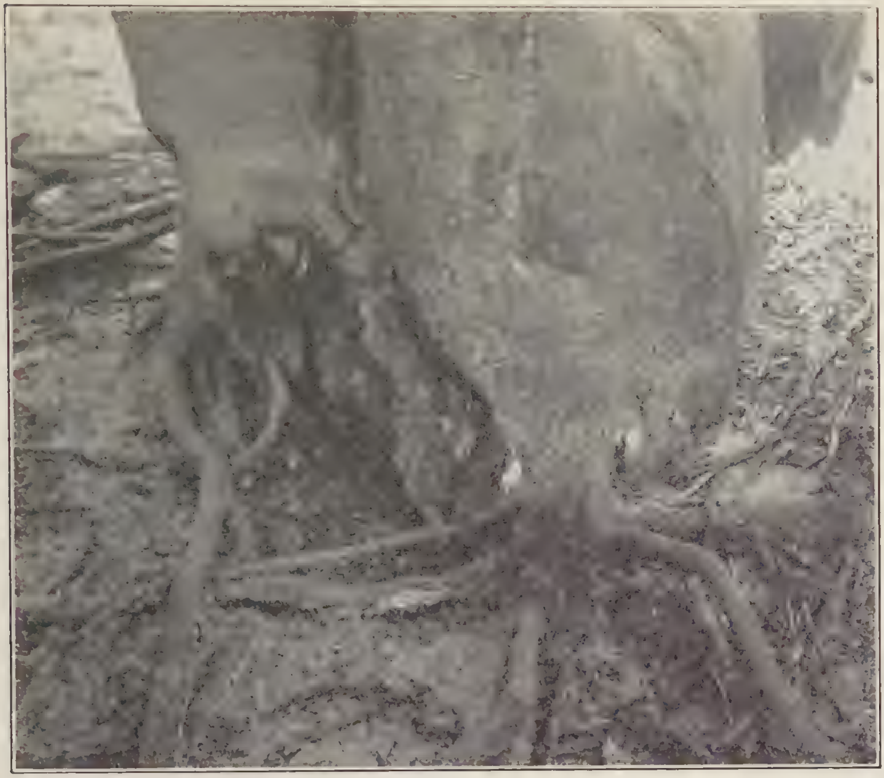

FIG. 1. New roots produced by banking old seedling grapefruit tree nearly dead from foot rot, showing how roots developed from margin of callus above old decayed areas have bridged the girdle and effected rejuvenation of tree.

of productivity to old, declining trees that were so badly girdled and rotted at the bases that they would soon have died or blown over. This method of treatment has been recommended in Florida for the past 15 years, especially as a last resort for trees that have declined too far to save by the usual methods of treatment.

The stimulation of the production of a new root system also has been found readily applicable to the so-called Australian pines (*Casuarina* spp.) attacked by Clitocybe root rot (*Clitocybe tabescens*). This disease works so insidiously in the *Casuarinas* that trees are often extensively girdled by the time a slight yellowing of the foliage branches becomes apparent on the lower limbs, which is the first symptom to manifest itself in the tops of the trees.

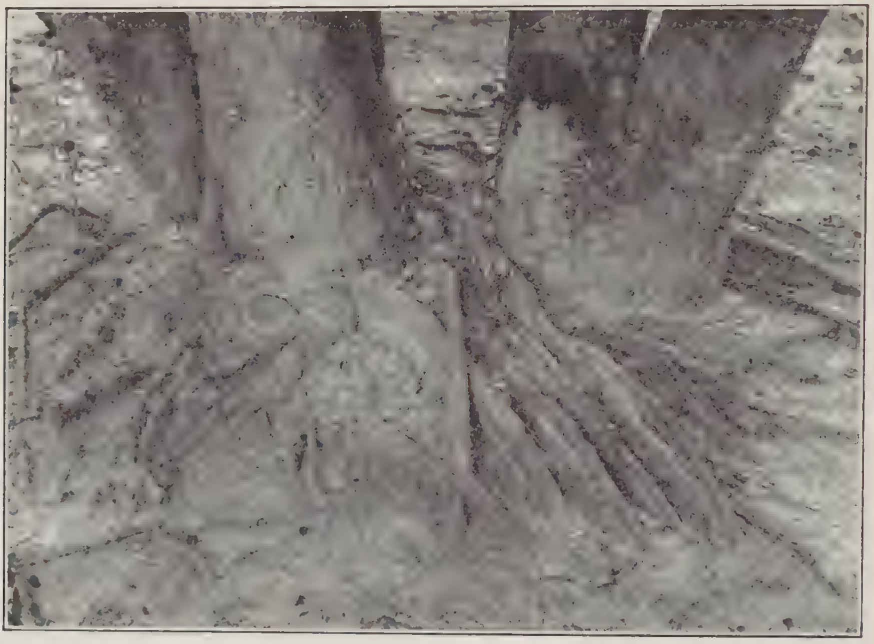

FIG. 2. Rejuvenation resulting from banking old seedling grapefruit tree nearly dead from foot rot, showing splendid development of new roots after partial washing away of bank.

The banking method of treatment was first tried on a tree of *Casuarina lepidophloia* that had been kept formally pruned to a cylindric shape, being one of several such trees in the yard of the writer's residence at Cocoa, Florida. When clipping this tree at the end of December, 1932, it was noticed that the lower limbs exhibited a slight pallor of the foliage branches and, at the base, a cluster of recently dried mushrooms of *Clitocybe tabescens* was found. Upon removing the soil to investigate the root system with a view to attempting to save the tree by surgical treatment, all the roots but three small lateral ones, of finger-size, on one side were found dead and invaded by the mycelium of this root-rot fungus. It was necessary to cut off and remove all the lateral roots but these, the base of the tree with the divisions of the taproot, and the basal bark, where the fungus had completely girdled the tree. The girdling even involved the 3 small lateral roots at the point where they left the root crown. All diseased bark was cut out down to the wood and the exposed wood surfaces were painted with a mixture of roofing paint and carbolineum. The tree was propped to hold it in position, but was blown to the ground the day following the treatment and had to be guyed into position with wire.

It was realized that this tree could not survive after this drastic treatment without further help. It was thought that if a new root system could be induced to develop from the margin of the living bark above the completely girdled base before the 3 remaining small girdled lateral roots ceased to conduct sufficient water to keep the tree alive there might be a remote possibility of saving it. A well of concrete blocks about a yard square was laid around the tree, filled with sandy soil, and watered. Following a prolonged drought, this tree lost a large proportion of its foliage branches in June, 1933, and looked as though it were going to die. However, occasional watering enabled it to survive, and, following the development of new roots, it began to improve after several months. By the middle of 1934 these new roots had so developed that the tree was as firmly anchored as it was originally. The well of blocks, therefore, was removed.

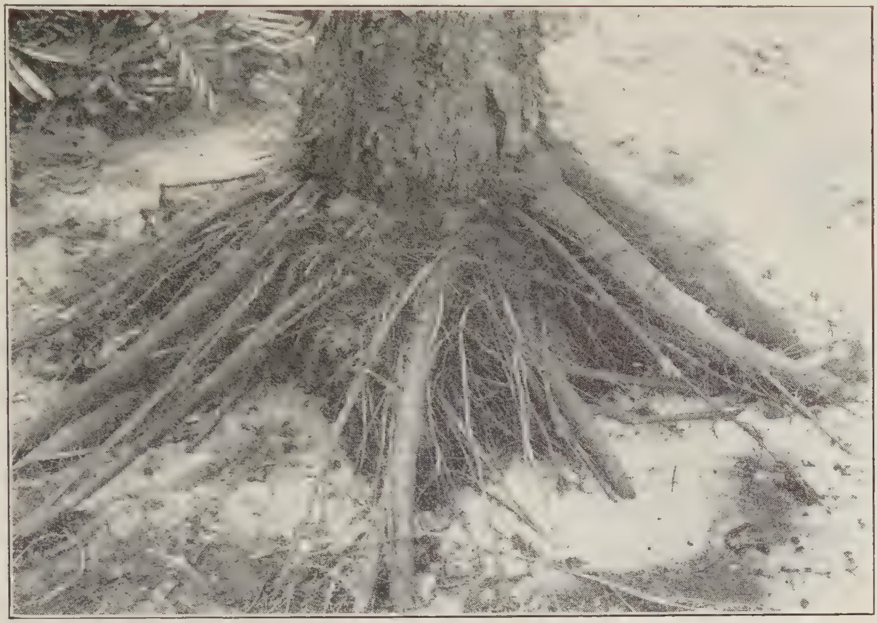

FIG. 3. New root system developed 4½ years after surgical treatment and banking of Australian pine (*Casuarina lepidophloia*) so nearly dead from Clitocybe root rot that all roots except three small lateral ones of finger-size on one side were removed and guying was required to hold tree in position.

When clipping this tree again in late April, 1937, the foliage branches on the lower limbs were observed to have become distinctly yellow again; investigation showed that the tree had become two-thirds girdled by Clitocybe root rot. The remaining mound of sand was washed away with a hose on May 16, and figure 3 shows the luxuriant development of new roots at this time.

The tree was re-treated by cutting away all diseased areas of bark on the base of the trunk and more than half of the newly developed root system. After painting the exposed areas of wood, 2 lb. of hydrated lime were scattered around the base and the sand was again banked around it. The tree soon regained its healthy appearance and has continued to remain healthy. When the bank of sand was washed away in June, 1941, to permit examination, a new growth of healthy roots, developed from the callus formed above where the bark was cut away on the trunk, was found to have filled in the gap left by removing the diseased roots approximately 3 years previously. Thus, by the simple expedient of banking, following surgical treatment, it has been possible to restore this apparently doomed tree to its former health and to continue to maintain it for a period of more than 9 years, despite reinfection by Clitocybe root rot. By so doing, it has been possible to preserve the ornamental value of a row of formally pruned trees in which the loss of a central one would have proved highly objectionable.

It is not known how this tree became infected again, as it was thoroughly treated at the end of 1932, when a small piece of oak root infected by the Clitocybe root-rot fungus was found under it and removed. One other formally pruned Australian pine similarly treated subsequently for Clitocybe root rot also became reinfected after the development of an excellent new root system was induced by banking following surgical treatment, but had declined to such an extent when the disease was observed that re-treatment and banking failed to save it. However, drought was an important factor in its death. Other trees of *Casuarina lepidophloia* attacked by Clitocybe root rot, including both formally pruned and unpruned ones, have been saved, without evidence of reinfection, for periods of several years by surgical treatment followed by banking. Cutting back the tops of treated trees was not necessary, even on unpruned ones.

In resorting to the banking method of treatment to save trees attacked by root diseases, it is advisable to treat the trees before they become girdled. On January 29, 1936, a far-fetched attempt was made to save another formally pruned tree of *Casuarina lepidophloia*, observed to be completely girdled by Clitocybe root rot 2½ months previously. In treating this tree it was necessary to excise the bark entirely around the base of the trunk up to a height of 18 inches on one side and 27 inches on the other. The entire root system was found to be dead from invasion by the fungus, but some roots had to be left to support the tree. A well of bricks about a yard square was laid around the base of the tree and filled with sand to a point well above the highest excised bark. This tree began to shrivel and die by March 15, and on March 30 the bricks were removed and the bank torn down to examine the extent of new root development. Several masses of tender roots, some as much as 12 inches long, were found to have developed from the lowest margin of callus formation and a few straggling ones higher up on the trunk in the period of two months following surgical treatment and banking. This, of course, was an extreme case and is cited to show the extent of root development that may be expected in a short time, even with the tree nearly dead.

The rejuvenation by banking of attacked trees following surgical treatment is by no means limited to citrus trees and Australian pines. It has been tried with considerable success by the writer and growers whom he has had occasion to advise, on a variety of trees, both large and small, and also on shrubs attacked by Clitocybe root rot. The results here reported have been secured under extremely droughty soil conditions and it is believed that even more favorable results would be secured in soils where favorable moisture conditions prevail. The method appears to be applicable to any woody plant that develops adventitious roots readily.

DISCUSSION

Surgical treatment or aeration, or the use of these in conjunction, is effective in arresting the progress of the disease. However, while such procedure may eradicate the infecting fungus, it does not assist trees handicapped by the loss of a large portion of their root systems to recover. The value of the soil-banking method of treatment is, therefore, apparent, as shown by the examples cited. In addition, it soon reduces or eliminates the possibility of the trees blowing over. It is essentially a natural method of inarching which has proved far superior to the usual method. It is possible that the use of some hormone-like substance to stimulate root development might be desirable, though excellent results have been secured on a variety of trees and shrubs thus far treated without recourse to such agents.

Soil-banking is diametrically opposed to the aeration method for control of root diseases. Since banking tends to favor the continued development of such diseases, it is a wise precaution ordinarily to first eradicate the disease by surgical treatment and disinfection and follow this by brief aeration. This is particularly advisable in the case of the mushroom or toadstool root rots, which are more difficult to control than other root diseases. In the control of foot rot of sweet seedling orange trees, aeration alone has proved very effective, since it appears to attack merely the bark. In many cases the disease appears to run its course after a time but the trees become weakened as a result of invasion by various, purely secondary, wood-rotting fungi. In long-standing cases of this disease on old trees, there are so many dead and rotted roots that no longer harbor the pathogenic organism, and so much decay in the bases of the trees, that painstaking surgical treatment is impracticable. With such trees, which would soon succumb or blow over ordinarily, the simple expedient of banking has been found to greatly prolong their life and productivity.

SUMMARY

The present well-known methods for the treatment of trees attacked by root diseases are discussed and evaluated.

Attention is called to the usefulness and practical value of a little-known natural method of inarching whereby trees and shrubs extensively girdled by root diseases may be rejuvenated by soil banking, either alone or in conjunction with previous surgical treatment, disinfection, and aeration, to stimulate the development of a new root system above the partially girdled bases.

The results secured by the use of this method on citrus trees and Australian pines are described and illustrated and its applicability indicated for use on woody plants that develop adventitious roots readily.

This soil-banking method offers the only known practical means of saving old sweet seedling orange trees in which the disease appears to have run its course and which have deteriorated to such an extent that painstaking surgical work is not justifiable.

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MOSAIC OF CELERY CAUSED BY THE VIRUS OF ALFALFA MOSAIC

William C. Snyder and Saul Rich

(Accepted for publication September 24, 1941)

In 1937 the writers observed that healthy plants of celery (*Apium graveolens* L.), artificially inoculated in the greenhouse with the virus of alfalfa (*Medicago sativa* L.) mosaic, developed a bright yellow mottle of a calico-like mosaic. Since that time, repeated inoculations have been made using alfalfa-mosaic virus of several sources collected from different areas in California, with the same kind of results. The writers make no attempt here to identify this mosaic with celery calico (1, 2), pseudocalico (3), or any other mosaic of celery previously reported, but describe it in the belief that it may be one of those involved in the complex of naturally occurring celery viroses. It is recalled that Black and Price (8) recently demonstrated that calico mosaic of potato may be caused by the virus of alfalfa mosaic.

Zaumeyer (4) apparently did not include celery in his host-range studies of the alfalfa-mosaic virus, nor did Price (5) report any test of this virus on celery in his comprehensive investigation of host ranges.

Four California sources of the alfalfa-mosaic virus were used in producing mosaic of celery in greenhouse studies on the host range of the virus. These inocula were obtained from field specimens of alfalfa growing in the Sacramento Valley, the Salinas Valley, and near Santa Maria and Riverside, respectively. Each of the original infected plants showed typical symptoms of alfalfa mosaic of the type illustrated in figure 1, A. The properties of the virus, determined for one of the collections, was found to be as follows: temperature inactivation point, 60–65° C.; dilution end point, 1/2000–1/3000; longevity *in vitro*, 3–5 days. The aphis transmissibility of the virus was demonstrated by means of the pea aphid, *Illinoia pisi* Kalt.

Infection was obtained by juice inoculations with the aid of carborundum as an abrasive, after the method of Rawlins and Tompkins (9). The amount of transmission obtained was usually low, but in a few trials it ranged as high as 50 per cent or more. Celery of the Golden Self Blanching type was used throughout. The inoculum for these trials was prepared by extracting the juice from *Vicia faba* L., *Petunia hybrida* Vilm., *Trifolium repens* L., and *Melilotus indica* All., respectively, artificially infected in each case by the alfalfa-mosaic virus. Successful transmission of the virus from celery to celery also was accomplished. The recovery of alfalfa-mosaic virus from celery was generally easier than its establishment in celery, and was obtained either systemically or in local lesions by mechanical inoculation of such host plants of the virus as *Medicago sativa* L., *Vicia faba*, *Vigna sinensis* Endl., *V. sesquipedalis* Wight, *Glycine max* Merr., *Petunia hybrida*, *Lathyrus odoratus* L., and *Trifolium repens*. The symptoms of the recovered virus

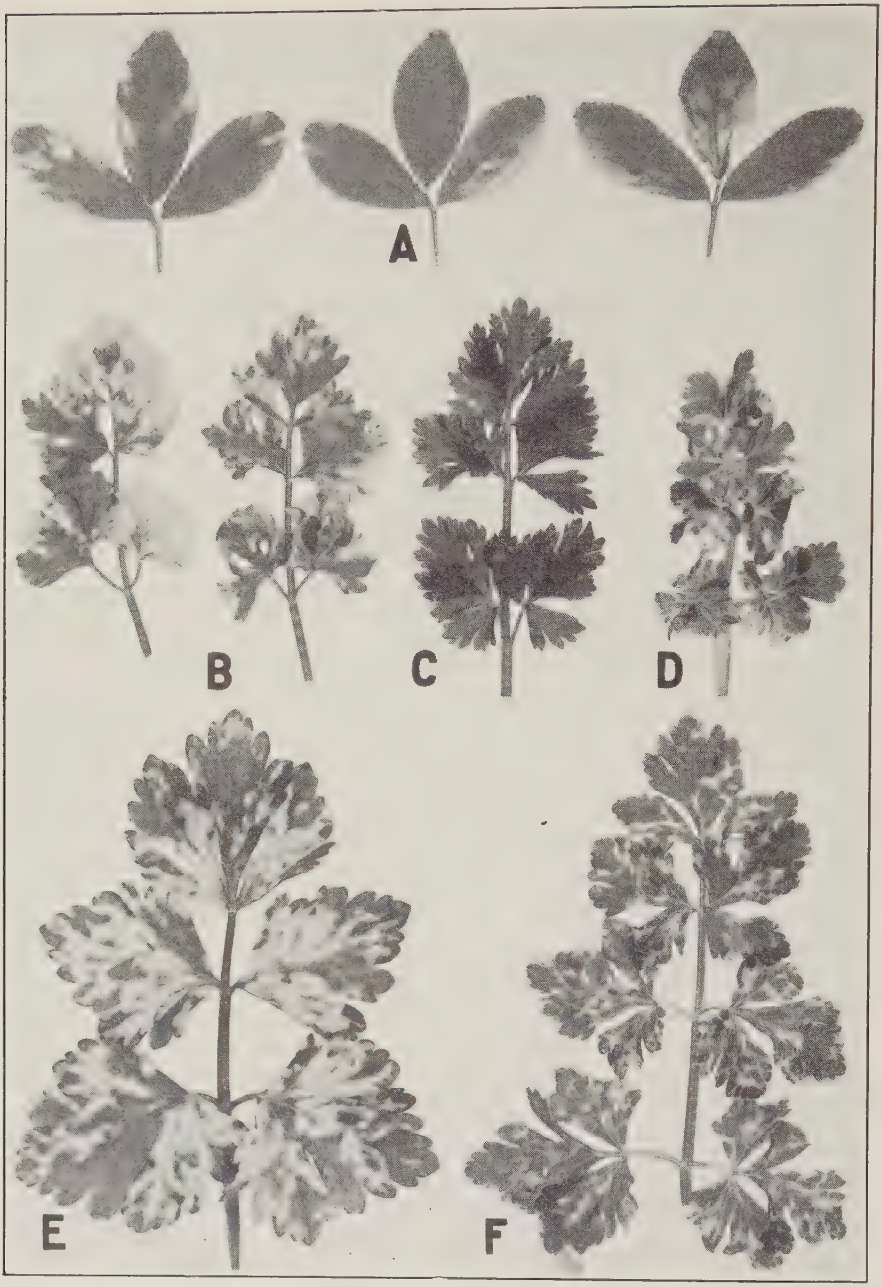

FIG. 1. Symptoms of alfalfa mosaic virus produced by artificial inoculation on common alfalfa, A, and on celery, showing mosaic on outer leaves, B, E, and F, and on an inner leaf, D. The leaf shown at C is from a healthy plant.

on these plants were the same as those obtained before its passage through celery, and, on alfalfa, were typical of alfalfa mosaic (Fig. 1, A).

The symptoms induced by alfalfa-mosaic virus on celery (Fig. 1, B F) consist of a mild to conspicuous yellow-green mosaic, principally of the outer leaves. In some cases, however, the inner leaves also show symptoms. When the symptoms are most marked, the affected leaf presents a striking calico-like pattern of lemon yellow patches on a normal green background. A mild blister effect often is associated with the occurrence of green islands of tissue in the yellow areas; and, in severe cases, a tendency to leaf distortion or a backward roll of the leaf may be observed. In early stages of the disease, vein clearing or a yellowing of the veins is often apparent. In later stages, yellow or cleared rings and halos may occur, surrounding areas of green tissue.

The appearance of symptoms on inoculated celery was sometimes slow, some inoculations requiring a month before the symptoms were well defined under the conditions obtained in the greenhouse. Sometimes, after 3 or 4 months from the time of inoculation, the symptoms faded out or changed to a general chlorosis, but the virus was still recoverable from such plants. A similar phenomenon was reported by Porter (6, 7) for potato calico and is well known for alfalfa mosaic.

It is suggested that there may be a direct relationship in the field between alfalfa mosaic and the calico-like mosaic of celery described here, especially when celery is grown in the vicinity of diseased alfalfa. Circumstantial evidence in support of this view is found in the observation that a calico-like mosaic frequently is found on celery in areas in which alfalfa is widely grown.

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PHYTOPATHOLOGICAL NOTES

The Perfect Stage of Phomopsis vexans.—The fungus, *Phomopsis vexans* (Sacc. and Syd.) Harter, has been known since 1912[1] to cause one of the most important diseases of eggplant (*Solanum melongena* L. var. *esculentum*). This pathogen and the disease, variously known as tip over, leaf blight, fruit rot, leaf spot, stem blight, and eggplant blight, that it causes have been studied by numerous investigators, among whom were Sherbakoff,[2] Edgerton and Moreland,[3] Nolla,[4] Palo,[5] Toole, E. H. *et al.*,[6] and Howard and Desrosiers.[7] It has never been reported present on any other host plant.

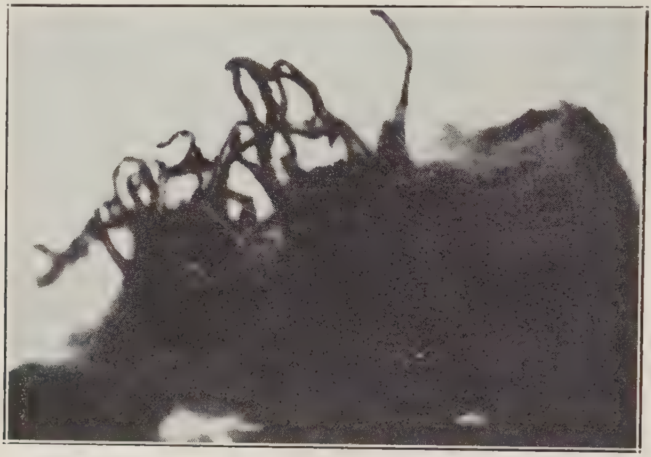

FIG. 1. A stroma bearing clustered, beaked perithecia of *Diaporthe vexans* growing on 2 per cent potato-dextrose agar. ×17.5.

To date the perfect stage of this fungus has not been described, but mycologists have long suspected that eventually it would be included in the genus *Diaporthe*. This is indicated by Wehmeyer as follows: "The imperfect or *Phomopsis* stages of several species of *Diaporthe* are known . . . to be the cause of serious diseases of several plants: for instance, *D. Citri* (*Phomopsis citri*) . . . ; *D. phaseolorum* . . . ; *D. sojae* . . . ; and *D. batatatis*. . . .

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[1] Harter, L. L. Fruit-rot, leaf-spot, and stem-blight of the eggplant caused by *Phomopsis vexans*. Jour. Agr. Res. [U.S.] 2: 331–338. 1914.

[2] Sherbakoff, C. D. Report of the Assistant Plant Pathologist. In Fla. Agr. Exp. Stat. Ann. Rpt. 1915: 94–98; 1916: 80–98; 1917: 76–86; 1918: 68–78.

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[5] Palo, M. A. The *Phomopsis* disease of eggplant and its control. Philipp. Jour. Agr. 7: 1–15. 1936.

[6] Toole, E. H., R. E. Wester, and Vivian K. Toole. The effect of fruit rot of eggplant seed germination. Proc. Amer. Soc. Hort. Sci. 38: 496–498. 1941.

[7] Howard, F. L. and Russel Desrosiers. Studies on the resistance of eggplant varieties to Phomopsis blight. Proc. Amer. Soc. Hort. Sci. 39: 337–340. 1941.

In these species the perithecial stage is rarely found or is known only from culture" (p. 3).[8]

In November, 1939, while making comparative studies of several isolates of *Phomopsis vexans* growing on 2 per cent potato-dextrose agar, it was observed that one of these was producing characteristically beaked structures not so superficial as the usual loosely gregarious pycnidia characteristic of other isolates on the same media. About 2 weeks later, and when the cultures were 6 weeks old, perithecia were observed embedded in the carbonaceous stromatous tissue, which was likewise partially embedded in the agar. This was again observed on another isolate in November, 1941.

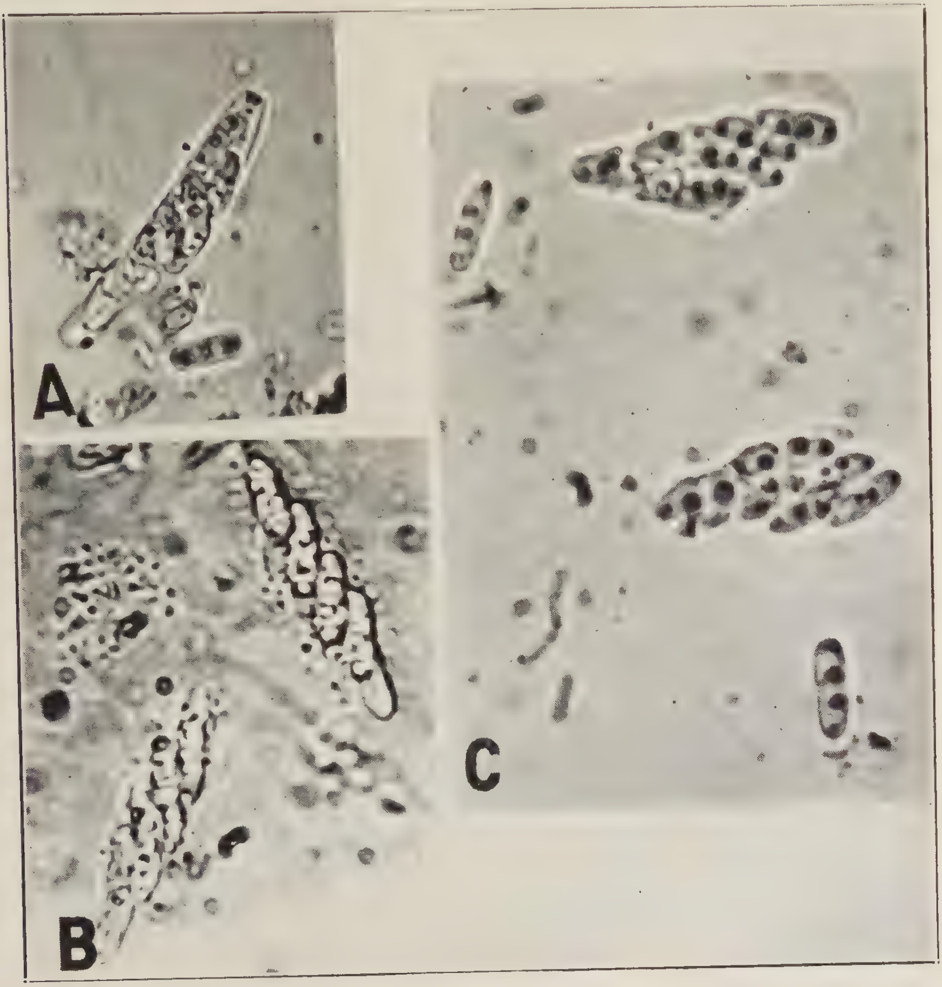

FIG. 2. Asci and ascospores of *Diaporthe vexans* showing: *A*. Apical pore. ×880. *B* and *C*. Guttulae, septation, constriction, bluntness, and arrangement of spores. ×920.

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[8] Wehmeyer, L. E. The genus *Diaporthe Nitschke* and its segregates. Univ. of Michigan Press, Ann Arbor. 349 pp. 1933.

These perithecia, occurring usually in clusters, were from 130 to 350 μ in diameter. The beak-like structures, ostioles, were carbonaceous, sinuate, irregular and from 80 to 500 μ long (Fig. 1). The asci produced in these perithecia were 8-spore, clavate, sessile, 28–44 × 5–12 (av. 36 × 8.9) μ, hyaline, with thin walls, and apex slightly thickened and pierced by a narrow pore. The spores were biseriate, hyaline, narrowly ellipsoid to bluntly fusoid, quite uniform in size, 9–12 × 3.0–4.4 (av. 10.8 × 3.7) μ, bicellular, constricted at the septum with each cell usually containing two guttulae (Fig. 2). In all respects, this fungus, isolated on two occasions from eggplant stems exhibiting characteristic symptoms of "tip over," from Marion County, Florida, is, in appearance, a typical *Diaporthe*. Fifty subsequent single-spore cultures from the same strain produced similar perithecia bearing asci and ascospores identical with those observed earlier.

Numerous lots of eggplant seedlings were inoculated with suspensions of crushed perithecia, or with ascospore suspensions made from the exudate taken from the tip of the elongated ostioles of perithecia, produced in the single-spore cultures just mentioned. Characteristic lesions with typical pycnidia on leaves and stems resulted from these inoculations; these were similar to those produced by parallel inoculations with pycnospore suspensions of *Phomopsis vexans*. Apparently, however, even though numerous, the spots and lesions resulting from inoculations with the perfect stage were not so abundant as those resulting from inoculations with the imperfect stage of the fungus. To date, the perithecial stage of this fungus has not been observed on the host plant.

In view of the evidence herein presented the binomial **Diaporthe vexans** (Sacc. and Syd.) n. comb. is proposed for the perfect stage of the fungus causing the "tip over" disease of eggplant.—L. O. GRATZ,[9] Department of Plant Pathology, Agricultural Experiment Station, University of Florida, Gainesville, Florida.

Influence of Temperature on the Expression of Big-vein Symptoms in Lettuce.—Big Vein, a disease of lettuce, was first described and named by Jagger and Chandler[1] who found it causing some loss in the Imperial Valley of California. While they stated that the cause of the disease was unknown, their observations indicated that the causal factor was soil-borne and persisted for extended periods in field and greenhouse soils. Since the appearance of their paper, no further investigations of the disease have been published.

In visiting lettuce fields in states along the Atlantic Seaboard during the last five years, the senior writer has observed in New Jersey, Maryland, and North and South Carolina what appeared to be big vein, and it seems probable that it may occur in other lettuce-growing sections in the East. At

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[9] The writer is indebted to Erdman West, Mycologist, Florida Agricultural Experiment Station, for helpful suggestions relative to this work.

[1] Jagger, I. C., and Norman Chandler. Big vein of lettuce. Phytopath. 24: 1253–1256. 1934.

Beltsville, Maryland, the disease has frequently been noted in experimental lettuce plantings in both field and greenhouse.

During the fall and winter of 1939–40, big vein appeared on a number of lettuce plants in the greenhouse at Beltsville, where the development of the symptoms was such as to indicate that temperature definitely affects manifestation of the disease. The group of lettuce plants in which the disease appeared was grown in 10-inch clay pots and held in a section of the greenhouse where, during the day, the temperature was originally maintained between 65° and 75° F., while at night it ranged from 50° to 60°. Occasionally the day temperature exceeded 75°, but only for short periods. When the lettuce plants had reached the stage of seed-stem elongation, it was necessary to reduce the day temperature to 50° to 60° and the night temperatures to 45° to 50°, because of the requirements of other experimental material

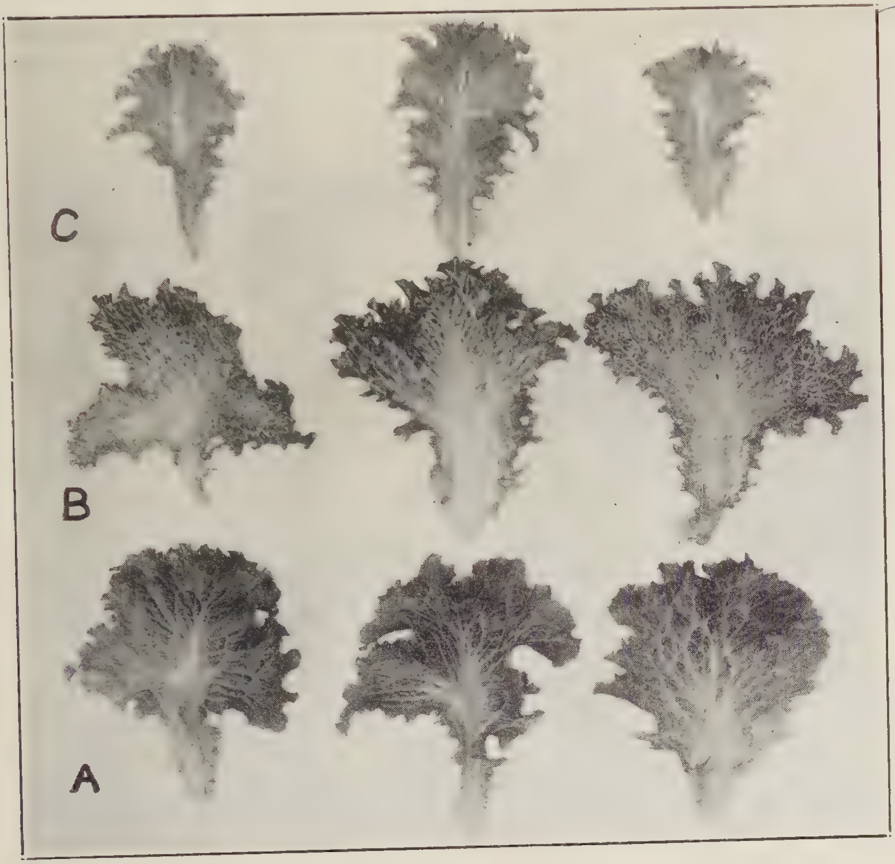

FIG. 1. Effect of temperature on expression of big-vein symptoms in lettuce. A. Leaves from base of seed-stem produced at fairly high temperatures (day, 65° to 75° F.; night, 50° to 60° F.) and showing no evidence of disease. B. Leaves from segment of stem directly above *A* produced at lower temperatures (day, 50° to 60° F.; night, 45° to 50° F.). These show pronounced big-vein symptoms. C. Leaves grown on segment of stem above B at same temperatures as A. Note that symptoms are again suppressed. (Photographed by transmitted light.)

grown in the same greenhouse section during this period. The temperatures thus maintained for 3 weeks were then raised to the original day and night ranges, and there held as nearly as possible until the plants reached maturity.

At the time the temperature was lowered all the plants were large and vigorous, of good color, and the leaves showed no evidence of disease (Fig. 1, A). At this time the seed stems of most of the plants had reached a height of 12 to 15 inches. During the succeeding 3 weeks at the lower temperatures, these stems made an additional growth of 8 to 12 inches. In certain of the plants all of the new leaves produced on that portion of the stems developed during the low-temperature period showed an enlargement of the vascular regions of the petiole and leaf blades. These areas were almost devoid of chlorophyll, and, when examined by transmitted light, a pronounced vein-banding was evident throughout the leaf. All of the affected leaves also were abnormally savoyed. These symptoms (Fig. 1, B) are all typical of the big-vein disease as described and illustrated by Jagger and Chandler[1] and it would appear that the plants showing such symptoms were infected before the temperatures were lowered.

When the greenhouse temperatures were again raised to 65° to 75° F. during the day and to 50° to 60° F. at night, the leaves first produced on those portions of the stem that developed after the temperature was raised showed some faint evidence of the vein-banding and savoying described above. As more leaves were produced, however, the later growth was normal in appearance and could not be distinguished from the foliage formed during the previous high-temperature period (Fig. 1, C). None of these later leaves showed any evidence of big-vein symptoms during the life of the plant, although the symptoms remained unchanged in the leaves of the low-temperature period.

These observations seem to indicate that the expression of big-vein symptoms in lettuce is greatly influenced by the air temperature prevailing at the time the leaves are in process of development. This belief has been confirmed by other observations on plants grown in the greenhouse and field at Beltsville, and is also supported by Jagger's report[1] that the symptoms of the disease were more pronounced and the percentage of diseased plants was possibly higher in crops grown in the Imperial Valley during the fall and winter months.—ROSS C. THOMPSON and S. P. DOOLITTLE, U. S. Horticultural Station, Beltsville, Maryland.

Sorghastrum, Host of an Undescribed Smut.[1]—The genus *Sorghastrum*, Indian grass, is abundantly represented throughout Virginia by *Sorghastrum nutans*, (L.) Nash. The long-awn Indian grass, *S. elliottii*, (Mohr) Nash, however, has been reported only in the Coastal Plain.

While on a fall collecting trip for vascular plants the senior writer found a small colony of *Sorghastrum* that showed a high incidence of head smut.

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[1] Cooperative Wildlife Research Unit, Dept. Biology, Virginia Poly. Inst. and Department of Plant Pathology, Agri. Extension Service, Penn. State College.

The location of the colony being in the Piedmont province, 125 miles west of the Coastal Plain, little attention was given to the species of the host. With attention focused on the infection, the host was arbitrarily accepted as *S. nutans*. Later, on examining the host, it was definitely identified as long-awn Indian grass, *Sorghastrum elliottii*, and thus the known range of the host species in Virginia was extended. The frequency of the occurrence of *S. nutans* over the State in contrast to that of *S. elliottii* probably explains why the smut had not been found previously, and especially, since host specificity is indicated. Host specificity is further indicated by the subsequent discovery of the disease on the same host in Georgia.

From the general appearance of the infection and the close relation of the host to *Sorghum*, the field impression was that of sorghum smut on a new host. When visiting Washington a few days later the material was shown to C. L. Shear and J. A. Stevenson of the Division of Mycology and Disease Survey. They expressed the opinion that *Sorghastrum* was a rare host for smut and that the smut probably was an undescribed species. Stevenson sent some of the material to the junior writer who reported that he not only considered *Sorghastrum* a rare host for smut but that the fungus was an undescribed species of *Sphacelotheca*. He was then asked to prepare a description of the new species for inclusion in this report. The name *Sphacelotheca sorghastri* is here proposed and described as follows:

Sphacelotheca sorghastri Zundel nov. sp.

Sori destroying the inflorescence, long linear, 7–10 cm. long, 3–4 mm. wide, at first protected by the leaf sheath, covered by a thick, yellowish membrane that flakes away, exposing a dark-brown, semi-agglutinated spore mass surrounding a compound columella of 7 or more branches extending the length of the sorus; membrane disintegrating into sterile cells, singly, in chains or in groups, globose to subglobose or ellipsoidal, flattened by mutual contact, hyaline, chiefly 7.5 to 14 µ diameter, vacuolated, thin epispore; spores globose to subglobose, regular, light olivaceous-brown, chiefly 7.5 to 10.5 µ diameter, abundantly and minutely echinulate, thin epispore.

On *Sorghastrum elliottii* (L.) Nash, Moses Mill Pond, 2 miles west of Chatham, Pittsylvania Co., Virginia. Coll. A. B. Massey. 5059 Sept. 5, 1941.

Latin diagnosis by Edith K. Cash of the Bureau of Plant Industry, U.S.D.A.

Sphacelotheca sorghastri Zundel nov. sp.

Sori inflorescentias destruens, longe lineares, 7–10 cm. longi, 3–4 mm. lati, primum vagina folii inclusi, membrana crassa flavida tecti; membrana frustulatim defrigens et massam sporarum nigrobrunneam, subagglutinatam denudans; columella e ramis 7 vel pluribus per longitudinem totam sori extensis compositis; membrana in cellulis sterilibus singularibus, catenulatis, vel aggregatis, globosis, subglobosis vel ellipsoideis, pressione inter se applanatis, vacuolatis, episporio tenui disrumpens; sporae globosae vel subglobosae, regulares, pallide olivaceo-brunneae, plerumque 7.5–10.5 µ diam., dense et minute echinulatae, episporio tenui.

In florescentiis *Sorghastri elliottii* (Mohr) Nash, prope Chatham, Virginia.

Since studying the material and describing the fungus as a new species, Zundel has received a specimen of the same smut on *S. elliottii* from Georgia. This was collected by Messrs. J. H. Miller and G. E. Thompson at Camp Willkins, Athens, Georgia, on October 14, 1941, thus indicating that the new species of *Sphacelotheca* is well distributed with its specific host.

The two species of *Sorghastrum* reported in Virginia are of little or no economic importance. In the Great Plains *Sorghastrum nutans* is an important constituent of tall-grass hay and is considered rather nutritious. In Virginia, however, it is not recognized as a hay grass. It occurs throughout the State, but usually only in small clumps or patches. The most extensive natural field development of this species has been observed in the Triassic area of northern Virginia, especially in Prince William and Fauquier Counties, where it occurs in fields of considerable acreage. It also has been observed on banks and slopes in several places in the State, effectively checking erosion. *S. elliottii,* on the other hand, has been reported previously in only a few Coastal Plain counties in Virginia; hence, it is of even less importance. The Sorghastrum smut, therefore, would be classed as of negligible economic importance. It does, however, present an attractive problem in the biology of a smut of a perennial grass.—A. B. MASSEY AND GEORGE L. ZUNDEL, Virginia Polytechnic Institute, Blacksburg, Va., and Pennsylvania State College, State College, Pa.

A Method of Mounting Cultures of Fungi for Preservation in the Herbarium.—It has been the custom in various institutions to file as herbarium specimens Petri-dish agar cultures of fungi. The agar removed from the dish is dried down on a sheet of paper or cardboard and then placed in a herbarium packet. This method has, at least, two disadvantages: (1) the agar sheet curls and shrivels, on drying, often becoming cracked or otherwise damaged; (2) the cultures thus mounted cannot be observed advantageously under the microscope by transmitted light.

Recently the writer has found that celluloid makes a more satisfactory mounting base than does paper or any of the other materials known to have been utilized previously for this purpose. Though the procedure followed in making such mounts is simple, it is outlined in detail below.

The celluloid[1] after being washed in soap and water, is rinsed, dried, and cut into squares of a diameter exceeding slightly that of the Petri dish. The agar medium with its mycelial mat[2] is lifted from the Petri dish with a spatula, laid in the center of the celluloid square, and allowed to dry. Within 3 days at room temperature ranging from 20° to 25° C., the agar is sufficiently dry to permit filing the mount in a herbarium packet.

The agar becomes firmly attached to the celluloid and, thereafter, will withstand a remarkable amount of twisting and bending. Mounts prepared in this manner were stored at relatively high temperatures (30–40° C.) for 10 days without the cultures cracking or peeling away from the base. However, the whole culture may peel off if the fungus is allowed to grow to the edge of the dish before being mounted. It is best to make the mount while there is still a peripheral strip of uncontaminated agar, at least ½ in. wide, around the thallus. If it is desirable to mount older or larger cultures, it may be necessary to apply first an adhesive or a fixative to the celluloid.

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[1] Obtained from the DuPont Viscoloid Co., Arlington, New Jersey, with the following specifications: 0.01 in. thick, transparent, color 7511, and HH finish.

[2] The fungi (*Stemphylium* sp., *Cephalosporium* sp., and *Colletotrichum* sp.) were growing on 2 per cent potato-dextrose agar, poured to a depth of approximately ¼ in. in the Petri dishes.

The cost of such celluloid bases is estimated at slightly less than one cent each (50 cents per sheet measuring 20″ × 50″).

Cultures preserved on celluloid can be examined by transmitted light under a cover slip if a drop of water, lactic acid, or other mounting fluid first be placed on the specimen. The gross characteristics, *e.g.*, zonation, chromogenesis, etc., can be studied almost as well as in the fresh condition.—M. B. LINN, Department of Plant Pathology, Cornell University, Ithaca, New York.

AMERICAN PHYTOPATHOLOGICAL SOCIETY
SUMMER MEETING

SECOR HOTEL, TOLEDO, OHIO

JUNE 25–26, 1942

In the rapidly changing times participation in war emergency meetings should be encouraged. The summer meeting of The American Phytopathological Society is definitely a war emergency meeting. It will deal with "The role of plant pathologists in a war emergency program." Emphasis will be placed on activities of the Society's War Emergency Committee. Everyone will be intensely interested in the progress made by this committee, and in assisting in formulating future activities.

Attend the summer meeting and be informed.

TENTATIVE PROGRAM

Secor Hotel
Toledo, Ohio

June 25

10:00 a. m. The role of plant pathologists in the war program. Summarized by the War Emergency Committee, E. C. Stakman, Chairman.

1:30 p. m. Round-table discussion on spray-material and spray-equipment priorities and substitute materials. This will include reports by members of the Society and representatives of industry. J. S. Horsfall, Chairman.

3:30 p. m. Demonstration of techniques used in determining physical properties of dust mixtures and performance of dusting equipment. Co-chairman, J. D. Wilson, Ohio Experiment Station, and Frank Irons, U. S. D. A., Engineering Laboratory.

7:30 p. m. Opportunity for regional or special committee meetings.

June 26

10:00 a. m. Discussion on extension, research and teaching policies during present emergency.—Leaders: H. B. Barss, O. D. Burke, N. E. Stevens.

1:30 p. m. Summary of program, policies and future activities of the War Emergency Committee. (Members of the executive committee in charge.)

Opportunity will arise in various sessions for a discussion of quarantines, the draft, disease surveys, and other subjects that the War Emergency Committee, or members desire.

An extensive list of Institutions, Societies, and Research Workers in the pure and applied plant sciences in C. and S. America has been prepared by the Editors of Chronica Botanica, in cooperation with the Div. of Agriculture of the Office of the Coordinator of Inter-American Affairs, Washington, D. C. It has been published in Chronica Botanica, Vol. 7, no. 2 and 3 (March and May, 1942).

IVAN CLAUDE JAGGER
1889–1939

DONALD REDDICK

The geological formations known as drumlins are a characteristic of the landscape along the southern border of Lake Ontario. Between these elongated hillocks were deep bogs which at the present time are intensively cultivated peat lands known throughout the region as muck. In a farmhouse at the base of one of these drumlins and overlooking a peat bog not many miles from Marion, New York, there was born on August 12, 1889, to Claude N. and Alvinette (Andrew) Jagger their first of four sons. He was named Ivan Claude.

IVAN CLAUDE JAGGER

The peat bog, which had supported a growth of mint of sufficient extent to support a still for the production of oil, was brought under tillage during the youth of Ivan; and on it was produced lettuce, celery, onions, carrots and other vegetable crops. Thus the technique of successful vegetable culture was ingrained by everyday observation and experience.

[VOLUME 32, NUMBER 7, JULY, 1942]

Early schooling was finished in 1907 after a course in the high school at Marion, a course devoid of science except for mathematics. It was at just this time that the State of New York had come to the support of higher education in agriculture by the expansion of a department of agriculture in Cornell University into a College of Agriculture with new buildings and an enlarged staff with L. H. Bailey as Dean. H. H. Whetzel came to the College in 1906, H. J. Webber in 1907 and B. M. Duggar in 1908.

On September 24, 1907, Ivan Jagger matriculated as a "regular" student in the College of Agriculture and undertook 19 hours of work including botany, chemistry, zoology, entomology, and two courses in mathematics. On the basis of excellent work, he was recommended for graduation at the end of the seventh semester in order to enter the Graduate School. The degree of B.S.A. was granted in 1911 and was conferred *in absentia*. Jagger was already in a field laboratory near Elmira, New York, engaged in a project for the improvement of the potato crop. The following year he undertook work on an Industrial Fellowship to investigate the downy mildew of onions, caused by *Peronospora destructor;* but when the disease failed to appear his time was fully occupied with a lettuce disease, the leafspot diseases of celery, with the smut of onions and a variety of other diseases of vegetable crops. The first two studies ultimately led to publications with the description of *Sclerotinia minor* and of *Pseudomonas apii* [*Phytomonas jaggeri* (Stapp) Magrou]. The work with *Urocystis cepulae*, the smut of onions, led to interesting developments and these were to be incorporated in a thesis to be presented in partial fulfillment of the requirements for a Doctor's degree. The preliminary work on this thesis was used as the basis for a Master's degree, which was granted at the University of Wisconsin in 1913 following a year of work there, but a permanent record in the form of a thesis was not made. In the spring of 1914 Jagger suffered a severe attack of pneumonia, which, coupled with severe chronic asthma, prostrated him for several months. The dissertation was never written and the studies of the smut organism are lost. The disease was just beginning to appear in these newer areas and by his timely work on smut control, Jagger unquestionably made it possible for thousands of growers to preserve their land for onion culture with negligible losses and at trifling expense. All of his work with onions is reported in 204 words in an Extension Bulletin treating of the diseases of vegetables, a bulletin which was compiled with much conscientious effort as a distasteful duty.

The studies of *Urocystis cepulae* were put aside for, in the meantime, July 1, 1914, Jagger had been appointed assistant professor in the College of Agriculture at Cornell and instructor in Biology at the University of Rochester, a cooperative endeavor by the two institutions in an attempt to see whether something could be done about the diseases of vegetables at Irondequoit, the vegetable garden for the city of Rochester. The "curl" of cucumbers was soon demonstrated to be a communicable disease and not a case of malnutrition, as had been supposed. His research was confined largely to the mosaic diseases of cucurbits, but his knowledge of diseases of vegetables and particularly his knowledge of how to be helpful without being obtrusive did a great deal to improve the plant sanitary conditions in this area of intensive crop production.

The conditions for work at Rochester were ideal in respect of research facilities, as well as the physical requirements of a chronic asthmatic, but in June, 1917, Jagger joined W. A. Orton's small army of inspectors and crop protectionists and was promptly sent to Florida where help was needed in the control of celery and lettuce diseases. It did not take long to develop a system of treatment which greatly improved the quality of the celery and the method is still employed in that area. Lettuce mosaic was investigated and a brief report made.

The asthma persisted and at Orton's suggestion Jagger went to Southern California in 1922, particularly in the hope that the climate would be beneficial to his health, but specifically to investigate an unknown disease of lettuce which was threatening the infant industry in the Imperial Valley. Jagger's own account of this "brown blight" disease has appeared posthumously in a recent volume of PHYTOPATHOLOGY but the practical results of his work are seen in the 8,000 carloads of lettuce which annually have moved out of the Valley to markets all over the United States. Both hybridization and selection within existing lines yielded highly resistant sorts, but the selection process in the hands of an experienced lettuce grower gave the practical result desired in a remarkably short time. In 1926, seeds were available to producers of seed. The growers had clamored for seeds in 1924, but Jagger insisted on one more year of testing. The clamor was then carried to Washington where a congressman tried to obtain seeds from W. A. Orton. Orton sent a telegram to Jagger and got back the reply, "one more year of testing." And so it was.

Upon releasing the lettuce seeds to producers, Jagger felt justified in taking the first vacation he had had since entering the profession. The year 1926 was spent quietly and with singular physical comfort studying, as an International Education Board Fellow, principally in the glass houses in England. But when he returned to California it was obvious that the work he had already instituted on the development of lettuce varieties that were immune to the mildew disease, caused by *Bremia lactucae*, must be pushed with vigor else the expanding acreage devoted to lettuce culture might have to be abandoned entirely. Furthermore, the powdery mildew of melons, caused by *Erysiphe cichoracearum*, was becoming increasingly destructive, and required attention. In 1930, in response to an inquiry, Jagger wrote as follows: "Prospects of a trip East for me are far in the distance. We are breeding disease resistant melons and lettuce, two generations of lettuce and three generations of melons each year, on limited funds and assistance. That leaves no time for anything except breeding melons and lettuce." A mildew-immune lettuce acceptable to the trade was produced only to have a new biological race of the parasite appear and make further work necessary. For the present the lettuce mildew is subjugated and at the time of his death the melon mildew appeared to be under control. The recent outbreak of mildew on melons strongly suggests the introduction of a new biologic race of this organism, and assuredly suggests the Jagger technique for overcoming it. It was Jagger's nonconforming isolate of *Colletotrichum lindemuthianum* that forced M. F. Barrus to further studies and the classic announcement that biologic races of this organism exist.

Ivan Jagger was a most congenial companion and no one ever could so much as question his absolute honesty and sincerity. Although he never held a teaching position, he was, in fact, very effective in the field of adult education. Just as he found time to give attention to dozens of minor diseases of vegetables, so in all of his experiments, he took opportunity to include some things which he considered to be demonstrations and to which he could lead interested growers. His contacts with his colleagues were relatively limited. During his student days he attended all of the meetings of our Society and made a contribution to the program. He also became a sustaining life member of the Society in those stressful days of 1916. He was a Fellow of the American Association for the Advancement of Science, a member of the Botanical Society of America, American Society for Horticultural Science, and a Fellow of the Natural History Society of San Diego. Much of his life was spent in a far corner of the country in a simple laboratory which he set up. Long trips by train in midwinter were a luxury with which he wisely dispensed, so that he was known intimately only by the few who happened also to be in the same far corner. Just as he was familiar with a great variety of diseases from personal study, so also he was familiar with the breeding possibilities of a number of plants. In his spare time and as a hobby he had collected and hybridized species of *Iris, Gladiolus, Brodiaea, Lycium,* and *Penstemon.* His knowledge of the wild progenitors of cultivated plants and of their relatives was very extensive, and his discussions of such topics came as a surprise to most of his friends.

Jagger had a most retentive memory. This stood him in good stead, for he disliked making and filing records. He knew the pedigrees of his selections without looking in the file and, if his successors would admit it, he almost certainly left selections for which no record exists. He also disliked the preparation of manuscript for publication. All of his articles are comparatively short, but they are direct, clear, concise, and adequate.

It is entirely characteristic that the portrait from which the engraving has been made is an enlargement from a small print made for passport purposes. It is a good likeness of fifteen years ago.

Death came at San Diego, California, on February 16, 1939. Surviving are his wife, Gertrude Fisher Jagger, two sons, Donald 1923–, and Paul 1932–, his father, and one brother.

PUBLICATIONS OF IVAN CLAUDE JAGGER

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A new biologic form of powdery mildew on muskmelons in the Imperial Valley of California. U. S. Dept. Agr. Plant Dis. Reporter **22**: 275–276. 1938. (With T. W. Whitaker and D. R. Porter.)

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The inheritance of immunity to mildew (*Bremia lactucae*) in lettuce. Abst. in Proc. Seventh Internatl. Congress of Genetics. Phytopath. **30**: 427–433. 1940. (With T. W. Whitaker.)

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THE RELATIVE EFFECT OF ENVIRONMENTAL AND GENETIC FACTORS ON GROWTH TYPES OF USTILAGO ZEAE[1]

M. F. KERNKAMP[2]

(Accepted for publication October 20, 1941)

When chlamydospores of *Ustilago zeae* (Beck.) Unger germinate, they ordinarily produce sporidia on their promycelia, but sometimes hyphal branches are formed instead of sporidia. The sporidia are easily isolated with a micromanipulator and, if allowed to grow vegetatively, will produce monosporidial lines. Many such lines have been isolated for the purpose of studying variability in this fungus. A phenomenon that complicates the study of variability in monosporidial lines of *U. zeae,* however, is the subsequent growth types of the resulting colonies. Unless hyphal branches are cut off, all colonies begin as single sporidia. The subsequent colonies may be entirely sporidial, entirely mycelial, or they may contain different proportions of sporidia and mycelium.

In a small percentage of cases the monosporidial isolates will continue to bud indefinitely and form colonies consisting entirely of sporidia. Such lines are designated as sporidial lines. If hyphal branches are cut off, they will produce colonies consisting of mycelium on potato-dextrose agar containing 1.5 per cent dextrose; but even some of these will form many sporidia on potato-dextrose agar containing 20 per cent dextrose. The lines that form no sporidia on agar with a 20 per cent sugar content are designated as mycelial lines. Since, however, strictly sporidial and mycelial lines occur very rarely, more attention will be given to those lines that change from a sporidial to an intermediate type of growth.

The change usually occurs during the first two months the lines are in culture, sometimes within the first 24 hours, but occasionally they change even after being in culture a year or more. The change generally occurs gradually, first becoming evident by the appearance of mycelial patches or pie-shaped mycelial sectors in the colonies. Several of these patches or sectors may appear simultaneously and thus all look alike, or they may appear at different times and, because of their different ages, look entirely different from each other. The question then immediately arises as to whether the latter constitute a genetic variation or whether the change from sporidial to an intermediate type of growth is temporary and phenotypic.

During studies on the variability of *Ustilago zeae* in the laboratories of the Division of Plant Pathology, University of Minnesota, many such patches and sectors have been isolated and compared in order to determine if they were genetic variants or only phenotypic changes. In many cases they have been found to be the latter. These circumstances made it confusing and often impossible to determine the degree of variability of monosporidial lines, and it seemed reasonable that if the factors that determine these growth types were better known, one could distinguish between phenotypic and genotypic changes with a greater degree of safety and thus make more accurate conclusions regarding the variability of monosporidial lines. Thus a study was begun on the factors that determine the growth types of monosporidial lines of *U. zeae.*

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[1] Summary of a thesis presented in partial fulfillment of the requirements for the degree Doctor of Philosophy, granted by the University of Minnesota, June, 1941. Paper No. 1926 of the Minnesota Scientific Journal Series.

Assistance in the preparation of these materials was furnished by the personnel of the Work Projects Administration, Official Project No. 65–1–71–140, sponsored by the University of Minnesota, 1941.

[2] Assistant Pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, U. S. Department of Agriculture. Formerly, Instructor, Division of Plant Pathology and Agricultural Botany, University of Minnesota.

In a recent paper on genetic and environmetal factors affecting growth types in *Ustilago zeae* (6), the following conclusions were drawn: (1) Growth types are genetically inherited; (2) strictly sporidial and mycelial lines probably cannot be changed by environmental factors; (3) intermediate lines can be shifted to mycelial or sporidial by certain environmental factors. The results also proved that these growth types are determined by at least more than one factor; and it was assumed that sporidial and mycelial lines carried factors only for sporidial and mycelial growth, respectively, and intermediate lines carried factors for both sporidial and mycelial growth.

In that study, however, only a few monosporidial lines were studied and relatively few environmental factors were taken into consideration. It seemed desirable, therefore, to investigate more thoroughly the environmental factors that might change intermediate lines, and to determine if sporidial and mycelial lines could be changed by environmental factors not previously considered. This paper, therefore, presents the results of a continuation of previous work on the relative effect of genetic and environmental factors affecting growth types of *Ustilago zeae.*

The literature on this subject has been reviewed adequately in a recent paper (6) and will not be repeated here. Since literature on specific phases of the problem is not extensive, it will be referred to where pertinent.

EXPERIMENTAL PROCEDURE AND RESULTS

Methods

All of the lines used in these experiments, except those from cross 50, originated from single sporidia isolated from promycelia by means of a micromanipulator, as described by Dickinson (3) and Hanna (4). The lines from cross 50 originated from hyphal branches cut from promycelia with a micro-razor.

It was necessary to use lines relatively constant for their particular type of growth. Lines, therefore, that had been sporidial, mycelial, or intermediate for several months, and, in some cases, years, were selected from a large number of stock cultures. Many intermediate lines contain sporidia but appear to be mycelial to the naked eye. In order, therefore, to know accurately the growth types of all of the lines it was sometimes necessary to examine them with a microscope. Having previously found that a high percentage of dextrose in the medium tended to increase formation of sporidia in intermediate lines (6), many of them were tested on potato-dextrose agar containing 20 per cent dextrose. Under these conditions one can distinguish between lines that will produce sporidia only with difficulty and lines that will not produce sporidia. The lines selected included 16 sporidial and 6 intermediate lines, and 1 mycelial line.

The designation of monosporidial lines indicates their origin. Thus, $70C_3$ is a monosporidial line isolated from the third cell of the promycelium of chlamydospore C of cross 70. Lines originating from hyphal branches were random isolates in that the chlamydospore and promycelial cell from which they came were unknown. Therefore, they were labelled with the cross number and a number indicating the order in which they were isolated. Thus, 50–2 is a culture which originated from the second hyphal branch isolate from cross 50.

The studies on the effects of environmental factors on the growth types of monosporidial lines were made with liquid media in hanging-drop cultures, with solid media in the form of agar smears on inverted cover slips, and with solid media in Erlenmeyer flasks.

In some cases a synthetic nutrient solution similar to that recommended by Brown (2) was used. It contained 20 g. dextrose, 2.5 g. asparagin, 2.5 g. tri-basic potassium phosphate, 0.2 g. magnesium sulphate, and water was added to make 1000 cc. This solution was varied so that different concentrations of dextrose were used, and where special substances were needed they were added in different concentrations to the basic medium. In the case of hanging-drop cultures a drop of each solution to be used was placed on each cover slip, which, in turn, was placed on a van Tieghem cell in a Petri dish, where the drops were kept from evaporating by means of filter paper placed on the bottom of the dish and moistened with distilled water. Five or 6 such drops were placed in each Petri dish, providing for as many replications.

When cultures were grown on solid media, potato-dextrose agar (1 or 2 per cent dextrose, 1.5 per cent agar, and infusion of 300 g. of potatoes for 1 liter of medium) was used in most cases. If agar drop cultures were employed, the procedure was the same as that described for the liquid hanging drops; if flasks were used, the cultures were grown in duplicate 250 cc. Erlenmeyer flasks containing 40 cc. of agar.

All similar media were prepared in one batch so as to have comparable media for each experiment. Each medium was prepared and sterilized in the autoclave for 20 minutes at 15 lb. pressure. In some cases special substances were added aseptically to the basic medium after it was sterilized.

EFFECTS OF ENVIRONMENTAL FACTORS ON GROWTH TYPES

Effects of Dextrose

Earlier experiments (6) had demonstrated that different amounts of dextrose do not influence the growth types of sporidial and mycelial lines of *Ustilago zeae*, but that intermediate lines can be made to produce greater numbers of sporidia on media containing high concentrations of dextrose and fewer sporidia on media containing low concentrations of dextrose. The cultures used in the earlier study were of lines that had been grown long enough to become stable so far as their growth types were concerned, but it seemed desirable to study some lines that were changing. Therefore, lines were obtained that had been isolated less than 1 month and were changing from sporidial to an intermediate type of growth in their first cultural generation. These cultures had patches of both sporidial and mycelial growth in the same colony, and transfers were made from both the mycelial and sporidial patches. The resulting cultures were treated exactly alike, and their labels were followed by (M) or (S) to indicate if they had originated from the mycelial or sporidial part of the colony. The following lines were obtained in this manner: $48H_2$(M), $48H_2$(S), $48C_4$(M), $48C_4$(S), $46F_1$(M), $46F_1$(S), $46T_4$(M), and $46T_4$(S).

The above lines were grown in hanging-drop cultures in the liquid synthetic medium containing 0, 20, 100 g. of dextrose per liter. Each culture was replicated 6 times and the experiment was repeated twice. The results are recorded in table 1. From the table it is clear that none of the cultures behaved exactly alike in these solutions, but the general trend of behavior was similar in all. All of them produced more sporidia in the solutions containing the largest amounts of dextrose. The cultures that began as mycelium, excepting $46T_4$(M), first formed some sporidia, and after different periods ceased to form sporidia and again became mycelial. Isolate $46T_4$(M) did not form sporidia even in the solution containing 100 grams of dextrose. In each case it took longer for the growth types to revert to mycelium on the higher than on the lower dextrose concentrations. The lines that began as sporidia generally behaved alike, except that more sporidia were formed in them than in those commencing as mycelium. One became entirely mycelial in the solution without dextrose, and some did not form any mycelium in the solutions containing a greater amount of dextrose.

A similar experiment was made with the same lines and the same medium, except that agar was added to make it solid. The results of the latter experiment were generally the same as the former in that larger numbers of sporidia consistently were formed on media with a high concentration of dextrose.

Since hanging-drop cultures do not become large enough to express such cultural characters as appear in flask cultures, it is impossible to detect any variations in color, topography, and types of margins resulting from mutations. Thus, there still is the possibility that the change in growth types observed in hanging-drop cultures may be attributable to undetectable mutations. These same lines, therefore, were grown in duplicate 250-cc. Erlenmeyer flasks on agar, in order to let their cultural characters

TABLE 1.—*The growth types of sporidial and mycelial parts of colonies of intermediate haploid lines of Ustilago zeae after growing for various periods in hanging-drop solutions containing different amounts of dextrose*

Monosporidial line	Amount of dextrose in medium																					
	None										2 per cent								10 per cent			
	Hours										Hours								Hours			
	12		48		60		72		96		50		72		80		96		83		108	
	M[a]	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S
$48H_2$(M) ...	+[b]	+	+	+	+	tr	+	0	+	0	+	+	+	tr	+	0	+	0	+	0	+	0
$48H_2$(S) ...	0	+	+	+	+	tr	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+
$48C_4$(M) ...	+	+	+	0	+	0	+	0	+	0	+	+	+	+	+	tr	+	0	+	+	+	0
$48C_4$(S)	0	+	0	+	0	+	tr	+	+	+	0	+	tr	+	+	tr	+	0	0	+	0	+
$46F_1$(M) ...	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0
$46F_1$(S)	+	+	tr	+	tr	+	tr	+	tr	+	0	+	0	+	0	+	0	+	0	+	0	±
$46T_4$(M) ...	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	±	0	+	0	+	0
$46T_4$(S) ...	+	+	+	+	tr	+	tr	+	tr	+	+	+	tr	+	tr	+	tr	+	0	+	0	+

Key: [a] M = mycelium
S = sporidial
[b] + indicates presence
0 indicates absence
tr = trace

develop fully. Potato-dextrose agar was used, containing 0, 20, and 200 grams of dextrose. The cultures were allowed to grow approximately 6 weeks after transfer to flasks; then they were examined both macroscopically and microscopically.

In this experiment the behavior of these lines was likewise generally the same as in the liquid hanging-drops and on agar drops. Generally there was more sporidial growth in the colonies on the media containing high concentrations of dextrose, and the colonies that began as sporidia all retained their sporidial growth on the medium containing 200 g. of dextrose per liter. To determine the permanence of the changes in growth type, reciprocal transfers were made from the 20 per cent to the 2 per cent dextrose medium, and from the 2 per cent to the 20 per cent dextrose medium. In all cases the cultures reverted to their original type and expressed the characters that appeared on the medium on which they were grown first. These results, therefore, indicate that mutations are not necessarily the causes of changes in growth types, since mutations are permanent changes. If the changes were due to mutations, it is not likely that the cultures would revert to the characters expressed on the original medium.

Effects of Extracts from "Natural" Substrates

Since the work of Brefeld (1), it has generally been accepted that the corn smut fungus will grow on such substrates as manure, soil, silage, and corn stalks, and that on these substrates aerial conidia are formed, which may be disseminated by wind. The writer attempted to determine to what extent soil, silage, manure, and corn extracts influenced the growth types of monosporidial lines of *Ustilago zeae.*

One mycelial, one sporidial, and several intermediate monosporidial lines were tested in hanging-drop cultures of these decoctions. Each culture was replicated six times and each experiment was repeated two or three times. Potato-dextrose broth was used as a check. The extracts were made sterile by filtering through a Sintered glass filter.

Soil Extract. Soil was obtained from the field plots at University Farm. One hundred cc. of distilled water and 100 g. of soil were mixed and allowed to stand so that the soil settled to the bottom. Then the water was poured off and filtered.

The soil extracts had no effect on the sporidial and mycelial lines, but almost completely prohibited sporidial growth in the intermediate lines tested. In potato-dextrose broth the intermediate lines were practically all sporidial growth. Since it is known that moisture stimulates sporidial growth (6), and soil extracts are obviously more liquid than soil, these results indicate that sporidia would not be formed in great numbers in soil in the field, except possibly in the case of strictly sporidial lines. Consequently, this would tend to decrease the possibility of dissemination of sporidia from soil by wind, but it would not decrease the possibility of the dissemination of aerial conidia.

Silage Extract. Piemeisel (10) reported that *Ustilago zeae* grew and formed sporidia in silage extract. In the writer's experiments, a silage extract was prepared as follows: 200 g. of silage, ground with a food chopper, were mixed with 200 g. of distilled water. After soaking for several hours the juice was expressed and filtered. This solution proved too concentrated to support growth, so it was diluted to one third of its original strength.

The same 3 haploid lines were grown in this solution; the mycelial line produced no sporidia; the sporidial line produced no mycelium; and the intermediate line became almost entirely mycelial. On potato-dextrose broth, the intermediate line was almost half sporidial and half mycelial. These results indicate also that if the fungus does grow on silage, only strictly sporidial lines would produce sporidia thereon.

Manure Extract. Fresh horse dung was obtained from the barns at University Farm. Equal quantities of manure and distilled water were mixed thoroughly; the resultant liquid was then expressed and filtered, and haploid lines of *Ustilago zeae* were grown in hanging-drops of the solution.

The growth types of the sporidial and mycelial lines remained unchanged in the manure extract. Five intermediate lines tested were entirely mycelial in the manure extract, but produced many sporidia in potato-dextrose broth. Another intermediate line produced a few sporidia in the manure extract, but in potato-dextrose broth it became almost all sporidial.

Corn Extract. It is obvious that *Ustilago zeae* forms hyphae in the host plant. Even lines that ordinarily form mostly sporidia in culture will form mycelium when injected into the host plants. However, several strictly sporidial lines have been found that have never caused infection in corn. It was, therefore, considered that these lines probably had no factors for mycelium formation, even in the host. It seemed desirable to determine if corn juice would change sporidial lines to mycelial, and, furthermore, if it would have any effect on intermediate and mycelial lines.

Fourteen-day-old corn seedlings were ground up with a food chopper and the juice was pressed from them and filtered. Hanging-drop cultures of a sporidial line, 2 intermediate lines and a mycelial line were grown in the extract. In the corn juice, the intermediate lines were almost entirely mycelial, while in the potato-dextrose broth, they were only approximately half-mycelial. In the sporidial and mycelial lines there was no apparent difference between growth in corn juice and in checks.

Another experiment was made in which corn juice was sterilized by autoclaving. In the autoclaved solution, there was slightly more sporidial growth in the intermediate lines than when they were grown in the filtered solution, but this solution had no effect on the mycelial and sporidial lines.

These results demonstrate clearly that corn juice stimulates mycelial growth in the intermediate lines, and add further evidence to indicate that strictly sporidial lines will not form mycelium, even in the host.

The results obtained from growing these lines on natural extracts may serve to indicate how intermediate lines behave in nature. Since nearly all lines are intermediate, it follows that most of them may behave as these results indicate. It is possible, however, that other soil organisms and various combinations of environmental factors in the soil may influence growth types. If few sporidia are formed on the substances tested, there obviously will be less dissemination of sporidia in the field from such sources as manure piles, corn stalks, and soil. This, of course, does not prevent spread of spores carried on manure or soil blown about by the wind, but it would decrease possible multiplication of sporidia and their dissemination from these sources.

Effect of Vitamin B_1 and "Growth Promoting" Substances on Growth Types

It has been reported by Schopfer and Blumer (11) that *Ustilago zeae* grows perfectly well on nutrient media without Vitamin B_1. Either the fungus does not require this vitamin, or it synthesizes it from the chemicals in the medium. However, it was considered by the writer that Vitamin B_1 might have some influence on the growth types of *U. zeae*, even though it is unnecessary for the nutrition of this fungus in synthetic media.

An experiment was made in which monosporidial lines of *Ustilago zeae* were grown in synthetic media containing 3, 30, 300, and 30,000 units of Vitamin B_1 per liter, but in no case were the growth types of the lines influenced by this Vitamin.

Along with vitamin B_1, two "growth promoting" substances, indole-3-n-propionic acid and tryptophane, were tested and found to have no effect on the growth types of *Ustilago zeae.*

Effect of Poisons on Growth Types

Earlier results (6) have indicated that conditions unfavorable to growth of *Ustilago zeae* tend to initiate in culture the formation of mycelium instead of sporidia. For example, an excess of magnesium sulphate in the medium decreased the rate of growth, as well as prevented the formation of sporidia, in intermediate lines. Such behavior was attributed to the toxic effects of the various chemicals when present in excessive amounts.

To test the validity of such an assumption an experiment was made in which the behavior of a sporidial, a mycelial, and 2 intermediate lines of *Ustilago zeae* were grown in nutrient solutions containing mercuric chloride, copper sulphate, lead acetate, and iron chloride. These chemicals were added to the basic solution mentioned earlier in the paper in the proportions of 1:500, 1:1000, 1:2000, and 1:5000, the basic solution without poisons being the check. Each culture was replicated 5 times and the experiment was repeated 3 times.

The mycelial and sporidial lines did not change when tested with the solutions containing toxic chemicals. The intermediate lines, however, produced fewer sporidia as the concentrations of the poisons were increased, and generally became entirely mycelial in the stronger solutions after 24 to 36 hours, and in the weaker solutions after 60 hours. There was little difference between the checks and the cultures in the solutions with the weakest dilutions of the poisons, but there was a striking difference between the checks and the cultures in solutions with high concentrations of poisons. These results, however, were somewhat erratic. For example, in the tests with copper sulphate at all concentrations, and with lead acetate 1:500, the intermediate haploid lines produced more sporidia when the cultures were approximately 24 hours old, and when they became 50 to 60 hours old they formed more mycelium and fewer sporidia. At 60 hours many of the sporidia became granular, stopped growing, and, consequently, did not germinate to form mycelium. As a result these cultures still contained many sporidia at the time growth ceased; therefore, they could not be considered truly mycelial at the termination of the experiment, as had been true with the other cultures. Nevertheless, it was clear that many sporidia were formed at the beginning of the experiment and, near the end of the experiment, all new growth was mycelial.

The results, as a whole, seemed to justify the general conclusion that toxic materials limit the formation of sporidia and increase proportionately the formation of mycelium in intermediate monosporidial lines of *Ustilago zeae.*

Effect of Dyes on Growth Types

Many organic dyes are toxic to fungi and in some cases are recommended for the control of certain plant diseases (9). Therefore, along with inorganic poisons, it seemed desirable to determine the effect of several dyes on the growth types of sporidial, mycelial, and intermediate lines of *Ustilago zeae.*

For this experiment malachite green, brilliant green, and crystal violet were added to potato-dextrose broth in the proportions of 1:1,000,000, 1:2,500,000, and 1:5,000,000. One sporidial line, 1 mycelial line, and 2 intermediate lines were tested. Each culture was replicated 6 times, and the experiment was repeated twice, potato-dextrose broth without dye being the check.

None of the dyes influenced the growth types of the sporidial and mycelial lines, and brilliant green did not appear to influence the growth types of the intermediate lines. The other two dyes limited the formation of sporidia in the stronger solutions. Malachite green was the most effective, and there were fewer sporidia in the 1:1,000,000 malachite green solution than in the lower concentrations. Brilliant green 1:1,000,000 appeared to be the only concentration of that dye that stimulated the formation of mycelium in the intermediate lines, growth in the other 2 concentrations being similar to that in the check.

Effect of Other Organic Compounds on Growth Types

Among chemicals tested for their therapeutic value in controlling plant diseases, para-toluene-sulfonylamide and ortho-toluene-sulfonylamide appear to be most promising (5). The effect of these two chemicals on the growth types of monosporidial lines of *Ustilago zeae* was tested.

In these experiments a sporidial, a mycelial, and an intermediate line of *Ustilago zeae* were grown in hanging-drop cultures. The nutrient solution consisted of these chemicals added to the basic synthetic solution in the proportions of 1:1000, 1:10,000, 1:100,000, and 1:1,000,000. Each culture was replicated five times, and the experiment was repeated twice. The basic solution with nothing added was the check.

Para-toluene-sulfonylamide and ortho-toluene-sulfonylamide had no effect on the growth type of any of the lines tested in these experiments, but there seemed to be some influence on the rate of growth. These chemicals at the concentration of 1:1000 definitely retarded the growth of the fungus, and ortho-toluene-sulfonylamide 1:1,000,000 seemed slightly to accelerate the rate of growth over the checks.

Effect of Carbon Dioxide and Oxygen Supply on Growth Types

It has generally been observed that when *Ustilago zeae* grows beneath the surface of liquid nutrient media it does not form sporidia, but the growth is characterized by narrow hyphae. Furthermore, if sporidia are formed in cultures growing in liquid media, they are invariably produced on the surface of the solutions. The oxygen supply in the solution and on the surface has generally been assumed to be the main factor involved in bringing about these different growth forms. Experiments, therefore, were made to test the effect of carbon dioxide and oxygen supply on the growth types of monosporidial lines of *U. zeae*.

The lines of *Ustilago zeae* were grown in hanging-drops of potato-dextrose broth, which were placed in atmospheres of approximately 50 per cent oxygen and 50 per cent air, 50 per cent carbon dioxide and 50 per cent air, 75 per cent oxygen and 25 per cent air, and 75 per cent carbon dioxide and 25 per cent air. Cultures grown only in air served as checks. As indicated in table 2, the growth types of the sporidial and mycelial lines were not changed by the different atmospheres of carbon dioxide and oxygen, but the rate of growth in the atmospheres containing 50 per cent and 75 per cent carbon dioxide was considerably reduced. A high carbon dioxide content in the atmosphere did not change the growth types of the intermediate lines but did retard the rate of growth. A high oxygen content of the atmosphere increased the amount of sporidial formation in the intermediate lines.

Effect of Osmotic Concentrations of Nutrient Solutions on Growth Types

There are a number of species of algae that have 2 distinct growth forms depending upon the culture media in which they are grown. One form is characterized by spherical cells that separate from each other after cell division; the other is a filamentous form. Livingston (7) reported in his experiments with a species of *Stigeoclonium*, that nutrient solutions of high osmotic concentration stimulated the spherical-cell form, and nutrient solutions of low osmotic concentration stimulated the filamentous form.

One can readily see the similarity between the growth forms of this species of *Stigeoclonium* and *Ustilago zeae*, and it seemed reasonable to suppose that similar reactions might occur in *U. zeae*. Although the results reported earlier in this paper indicate that changes in growth types are stimulated by the quantity or availability of the various food elements used, they did not preclude the possibility that the stimulating factor might be the osmotic concentration of the nutrient solutions. Experiments, therefore, were made to determine whether osmotic concentration of nutrient solutions would influence the growth types of *U. zeae*.

TABLE 2.—*The growth types of sporidial, mycelial, and intermediate haploid lines of Ustilago zeae in atmospheres containing proportions of carbon dioxide and oxygen to air*

Monosporidial line	50% CO_2		75% CO_2		50% O_2		75% O_2	
	M[a]	S	M	S	M	S	M	S
$18C_3$ (sporidial)	0[b]	+	0	+	0	+	0	+
50–2 (mycelial)	+	0	+	0	+	0	+	0
$22C_1$ (intermediate)	+	+	+	+	+	+	tr	+
$22B_2$ do	+	+	+	+	±	+	tr	+
$70C_3$ do	+	+	+	+	±	+	tr	+

Key: [a] M = mycelium
S = sporidial
[b] + indicates presence
– indicates absence
tr = trace
± approximately 25% mycelium and 75% sporidia in the cultures.

Livingston's experiments (7) were repeated, with only slight variation in the quantities of nutrients used, with *Ustilago zeae*. The osmotic concentrations of the solutions were calculated from the International Critical Tables of Freezing Point Depression of solutions, and checked by the freezing point method described by Loomis and Shull (8). Mycelial, sporidial, and intermediate lines were tested in solutions of different osmotic concentrations. In no case did these solutions influence the growth types of these lines. The experiment was repeated 3 times; the third time small but equal quantities of dextrose were added to the solutions. This only stimulated better growth; but, again, there was no difference in the growth types of any culture in the solutions of different osmotic concentrations.

EFFECTS OF GENETIC FACTORS ON GROWTH TYPES

Sporidial × Sporidial Matings

It has been mentioned previously that certain monosporidial lines have never formed mycelium in culture, either on potato-dextrose agar or under the different environmental conditions considered in these experiments.

Crosses had been made between some of these lines and mycelial lines, and there was segregation for sporidial and mycelial growth types in the progenies of these crosses (6). Likewise, successful crosses were made between sporidial and intermediate lines. Efforts were made to hybridize sporidial lines for the purpose of studying the inheritance of the sporidial character, but all attempts at hybridization were unsuccessful. Sixteen sporidial lines were paired in all possible combinations and injected into corn seedlings in the greenhouse, but in no case did infection result. This was repeated at least 10 times, but still no infection was obtained. The question immediately arose as to why these lines failed to cause infection when inoculated into corn seedlings. Two possible explanations for this behavior may be that all of the lines are of one sex and consequently sexually incompatible, or that the lines are so definitely sporidial that they cannot form hyphae in the host and, consequently, cannot fuse to form the dikaryotic mycelium.

The first possibility was eliminated by crossing some of the sporidial lines with tester lines of opposite sex. Table 3 gives the results of these matings.

TABLE 3.—*The compatibility of several sporidial lines with two tester lines of opposite sex*

Sporidial line	Tester lines of opposite sex	
	$22B_2$	$22B_4$
$18C_3$	+[a]	−
$18A_1$	−	+
$18H_1$	+	−
$18H_4$	−	−
$18A_4$	−	+

Key: [a] + = compatible
− = incompatible

Lines $22B_2$ and $22B_4$ are of opposite sex and cause infection readily. As is indicated in table 3, $18C_3$, $18H_1$, and $18H_4$ were all compatible with $22B_2$, and $18A_1$ and $18A_4$ were compatible with $22B_4$. Hence it follows that $18C_3$, $18H_1$, and $18H_4$ are of a different sex from $18A_1$ and $18A_4$. This was done also with several other pairs of monosporidial lines known to be compatible, and similar results were obtained.

The second possibility was tested by making histological studies of corn seedlings that had been inoculated with a composite inoculum of several strictly sporidial haploid lines. The seedlings were sectioned at the point of injection 24, 48, and 72 hours after the injections were made. The sections of the fresh tissue were stained with cotton blue or safranin and fast green. In the stained sections, numerous sporidia could be seen, but in no case were the sporidia observed to germinate and form hyphae. This was repeated many times and the results were always the same. Corn seedlings, inoculated with compatible haploid lines, were treated in the same manner, and mycelium invariably could be found in these plants.

It is possible, but hardly probable, that the mycelium could have been overlooked in the plants inoculated with strictly sporidial lines. The results indicate that these sporidial lines will not form mycelium inside the host plant, and, consequently, cannot cause infection when mated with each other. However, the strictly sporidial character can be perpetuated by combining these lines with intermediate or mycelial lines. It seems reasonable that sporidial and mycelial lines may combine by the growth of hyphae of the mycelial or intermediate line, so that it comes in contact with the sporidia of the sporidial line within the corn plant, and in this way fusion can occur.

SUMMARY AND CONCLUSIONS

These results are in agreement with those reported earlier (6), namely, that growth types of monosporidial lines of *Ustilago zeae* may be strictly sporidial, strictly mycelial, or intermediate between sporidial and mycelial. The growth types of sporidial and mycelial lines cannot be changed, but the intermediate lines can be shifted one way or the other by various environmental factors tested. Dextrose was the most efficient in stimulating the formation of sporidia in intermediate lines. Mycelial formation in intermediate lines in general was stimulated by environmental conditions that were unfavorable to the growth of the organism. For example, the addition of poisons and toxic dyes to media, low concentrations of necessary nutrients, and of oxygen in the atmosphere stimulated the formation of mycelium. Other substances, such as vitamin B_1, indole-3-n-propionic acid, tryptophane, para-toluene-sulfonylamide, and ortho-toluene-sulfonylamide, had no effect on growth types.

The high concentrations of sugars in some of the media indicated that the formation of sporidia in intermediate lines might be brought about by the high osmotic concentrations of those media. This possibility, however, was eliminated by experiments proving that the osmotic concentration of nutrient media was not the stimulating factor. All of the results indicate that the quantity of nutrients in the media is the most important factor in determining the growth types in intermediate lines in culture. Although some of the other environmental factors influenced the growth types of intermediate lines to some extent, their effects were not nearly so strong as those of nutrients; in fact, lines, recently isolated, could be retained in the sporidial condition by growing them on media with a high sugar content. Furthermore, after lines had changed to an apparently mycelial growth type, the writer was able to change many of them back to intermediate, and thereby distinguish between those lines apparently entirely mycelial and those almost so, but producing a few sporidia. Since the change from sporidial to intermediate type often can be prevented or reversed by environmental conditions, these results would indicate that in many cases it is due to physiological processes.

The experiments with "natural" substrates serve to indicate how *Ustilago zeae* may behave in nature. All of the "natural" substrates definitely limited sporidial formation in intermediate lines and stimulated mycelial formation. Virtually all lines of *U. zeae* are intermediate. Therefore, if these results do indicate what takes place in nature, it follows that most of the growth in nature would be in the form of mycelium. In this paper, however, no consideration was given to effects of other organisms in the soil or other soil factors, but it is highly probable that they might influence growth types also.

Stakman (12) reported clear-cut segregation for growth types in the promycelia of chlamydospores, and Kernkamp (6) reported segregation for growth types in the progeny of a cross between a sporidial and a mycelial line, thus definitely proving that growth types are inherited. Crosses between intermediate lines that are mostly sporidial have given rise to progenies that are mostly sporidial, and crosses between lines that are mostly mycelial have increased the prevalence of mycelial segregates in those progenies.[3] But efforts to cross strictly sporidial lines were always unsuccessful. After examining many sections of corn plants inoculated with combinations of sporidial lines and invariably failing to find mycelium in these plants, it was concluded that these lines could not cause infection when mated together because they are incapable of forming mycelium, even in the host. They are, however, pathogenic when mated with lines that form hyphae. This strengthens the general conclusion that the strictly sporidial growth types are limited by genetic factors and cannot be changed by environment.

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COACERVATES IN PHYSICAL AND BIOLOGICAL SYSTEMS[1, 2]

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Our previous studies on the effects of a nutrient deficiency in orange leaves, known as "mottle-leaf," called attention to certain vacuolar inclusions not found in cells of healthy leaves (13, 14). These inclusions were generally refringent in transmitted light and each was surrounded by a distinct layer that would absorb dyes, such as acid fuchsin, and could be plainly seen under the microscope. In the absence of more exact information at that time, we concluded that these spherical bodies contained phytosterol material or phospho-lipoids. The affected orange leaf cells gave evidence that there had been a decided shift in the oxidation-reduction equilibrium. Cells of the affected leaves could also be distinguished by their power to reduce methylene blue and Nile blue A.

These spherical inclusions were not confined to the leaves, but we demonstrated them in the post-meristematic cells of the vegetative buds of orange shoots on affected branches (12). The enveloping membrane and the tendency to clump were there clearly demonstrated, though at the time of the publication no physico-chemical interpretation of the inclusions was given. However, in a later publication we advanced the idea that these spherical refringent bodies represent suboxidized products of the metabolism of carbohydrates and proteins in these affected cells. In the case of the zinc-deficient cells, there was evidence that the sulfhydryl compounds, such as cystein, were stabilized by the zinc salts that were present.

We have been able to demonstrate the presence of these inclusions in living cells by the application of neutral red or methylene blue in low concentrations; hence we assured ourselves that we were not dealing with artefacts. Moreover, other investigators, employing their own techniques, have described analogous bodies in the cells of other plants.

We shall in this paper present further investigations on the nature of these characteristic inclusions in pathological plants.

RECENT STUDIES

Since a knowledge of the factors that affect the stability of hydrophilous sols is important for pathologists and physiologists, we shall mention a few recent contributions to the subject dealing with non-living systems. It is known that under certain conditions there may appear, in an hydrophilous sol, bodies in which colloids have become concentrated through a phenomenon that might be termed a "demixing," or a separation of phases (9) or, more precisely, a "coacervation" process (7). The coacervate is a body rich in colloids and immersed in a liquid relatively poorer in colloids, which has been designated the equilibrium liquid.

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[1] The work herewith reported was done in the Division of Plant Nutrition, University of California, Berkeley.

[2] Cost of publication of this paper was borne by the University of California.

Bungenberg de Jong and Kruyt (7) postulated that flocculation and coacervation are very closely allied phenomena, each requiring the elimination of the stability factors, charge and hydration. For example, in the presence of neutral salts, a polyphenol may induce either flocculation or coacervation, according to the temperature and viscosity (as determined by mucilages).

Coacervation results from a lowering of the "solvation" and a reduction of the charge on the particles that may become coacervated. To remain suspended or dispersed as an hydrophilous sol, each particle must be surrounded by its own "solvation mantle," meaning a peripheral zone of molecules of water in the solvation medium. The molecules of water nearest to the particle are oriented around it; farther away, the molecules are less and less distinctly oriented, so that they gradually become distributed at random in between the different "solvation mantles," as Bungenberg de Jong pointed out (6). The action of the desolvating agent reduces the large diffuse solvation mantle around each particle to a thin layer of oriented water molecules, which are sharply separated from the bulk of surrounding, randomly distributed water molecules. These oriented water molecules are surrounded by a boundary, which plays the role of an active layer. Following the diminution in thickness of the solvation mantle through the desolvation process, the particles may gather so closely that each layer of surrounding oriented molecules of water loses its individuality and the group of particles becomes coacervated within a common boundary which separates the "coacervate" as a whole from the surrounding equilibrium liquid. Hildebrand (5) concluded that one cannot make a definite distinction between the water acting as a solvent and that acting as water of hydration, since the solute is chemically bound to some of the water and this, in turn, chemically bound to water molecules farther removed.

First, we shall inquire what might happen if one would introduce into this structure solutes of different types—for example, ions with single or multiple charges; molecules with dipoles, or molecules, which, themselves, can participate in the formation of hydrogen bonds, because it seems logical to interpret a coacervate as the result of a disturbance of hydrogen bonds in the water solution through the agency of oxidases such as catechol oxidase. Second, we will investigate the process of vital staining, as a disturbance of hydrogen bonds through the introduction of electrically charged particles of the vital stains. Third, we shall interpret in the same light the significance of "hydrogen donors" as solvents of the coavervates.

Ethyl alcohol, methanol, acetyl acetone, acetone, and tertiary butyl alcohol were reported by Russell (15) to be good solvents of natural phlobatannins, which are understood to be oxidation and polymerization products of catechol. To these should be added ethylene glycol and dioxan (diether of glycol), methyl cellosolve, and tetra ethylene glycol dimethyl ether.

Among these solvents, those of special interest to us now are the "donor solvents," which are able to release hydrogen to the substance to be dissolved (or solute), thereby building a solute-solvent association by means of CH ← O bonds. Hydrogen donors as solvents have been studied by Zellhoefer, Copley, and Marvel (16) and by Copley, Zellhoefer, and Marvel (2, 3).

The biological importance of the phenomenon becomes significant when we pay attention to the correlation between oxidation of catechol and polymerisation as oxidation processes, and to linkages that unite molecules, building up molecular associations that may ultimately reach microscopic sizes. These are reversible phenomena, and introducing H into the system will both induce reduction and break up the molecular linkages, as H will satisfy many of the bonds, and compete with molecular linkages. Therefore, it was to be expected that hydrogen donors would act as solvents of the oxidized, polymerized and coacervated catechol colloids.

Complex micellar systems are those in which complex relations between micelles exist; that is to say, those in which an effective attraction of charges is opposed to the tendency to solvation of the components. "Complex" and "auto-complex" coacervates as defined by de Jong (6) form only in the particular cases of these complex colloidal systems.

These are of two kinds:

(a) the opposition of the charges is created by the charged colloids of opposite signs = complex colloidal systems in a strict sense;

(b) the opposition of the charges is created by adsorption or linkage of appropriated ions at the surface of a single species of particular colloids = auto-complex colloidal systems.

Among the different types of coacervates that can be conceived as physically possible, it is advisable to reconsider Bungenberg de Jong's (6) "auto-complex," in which the coacervation involves phosphatides and is irreversible. He emphasized its ability to induce persisting desequilibria between adjoining solutions. As the movement from outside or from within of any given solute would be determined by the "permeability," auto-complex coacervates would contain the elements each of which had been considered as responsible for cell permeability—namely, water, molecules of cholesterol or phytosterol, and a certain number of adsorbed calcium ions.

The equilibrium between the coacervate and the surrounding liquid may be altered by further lowering the solvation. This would retard the diffusion of some of the solvate liquid out of the coacervate. It is not to be denied that the change of equilibrium may be so abrupt that it provides no time for diffusion. When that state exists in the system, some of the solvating agent will separate out as "vacuoles" within the coacervate. When those drops are exceedingly small and numerous, they cause the coacervate to appear black under the microscope. The writers frequently found this to be the case in walnut leaves showing the effects of a deficiency of zinc.

Many of the coacervates present a notable resistance to high salt concentrations. For example the concentration of NaCl reaches 200 milliequivalents for some and may rise to 0.8 N at the time when it does not exceed

50 m.e. in complex coacervates of gelatin with gum arabic, and 12 m.e. with those of gelatin with egg lecithin. This fact argues strongly in favor of the hypothesis assigning to the complex coacervates a part in the constitution of living matter.

According to Klercker (8) polyphenols may occur in solution in some plant cells; in others they may occur as characteristic tannin-vacuoles, each with an enveloping membrane. He described the complex coacervates formed in the vacuolar sap of plants when cells were submerged in a hypertonic solution of KNO_3. The cause of the phenomenon may be referred to the desolvating action of the salt solution, which withdrew water from the cell vacuole and led to coacervation of the catechols. When the KNO_3 was washed out there was a disappearance of the coacervates due to the dispersion of the catechol. It can be termed an increase of "solvation," *i.e.*, mixing.

Molisch (11), who investigated crystalline and amorphous anthocyanins, found with surprising frequency the occurrence of anthocyanins as crystals or as amorphous aggregations in the vacuolar sap. The latter, which were generally globular, had tendencies (as shown by illustrations) to zonation. Molisch tacitly assumed that the crystals or balls in such cases consisted of a chemical combination of the pure pigment with another substance, possibly a tannin compound.

Massee (10) saw and described "tannin-vesicles" in the vacuoles of leaf cells of orchids affected with the "spot" disease, explicitly stating, however, that the vesicles do not consist entirely of tannin. He believed that in many respects they resembled the tannin aggregates described by Klercker. The illustrations accompanying his article show several types of vacuolate globules, which occurred in the cells of affected orchid leaves.

At present there can be no doubt that the globular aggregates formerly seen in plant cells by various observers were complex coacervates of catechol and lipoid materials.

Coacervates are porous systems or molecular sieves, inasmuch as the micellae are separated by the solvatant liquid. Lecithin, phytosterol, or other bodies accumulated in the solvatant membrane, are lipoids and their role in permeability was postulated by Overton. Since the solvatant membrane contains water, as well as the accumulated lipoids, it, therefore, seems possible to consider it as a mosaic of water and lipoid constituents, as assumed by Collander (1).

The main factor making possible the auto-complex coacervation of a phosphatid is a certain stage of desolvation. This could be achieved through a water-soluble desolvant, such as catechol or other polyphenols, though without any permanence, as these water-soluble desolvants would immediately be dispersed in the surrounding liquid, but the living cell also contains desolvants, *e.g.*, phytosterol, which are practically insoluble in water, though able to enter into complexes with water. The cytological significance of phytosterol has been previously emphasized by Guilliermond and Mangenot.

CYTOLOGICAL INVESTIGATIONS

It is the purpose of the present paper to present some data obtained from the study of plant roots and to relate certain pathological aspects to the supply of mineral nutrients. We shall emphasize the abnormal organization of the root cells with especial reference to the presence of globular aggregates and discuss the processes of coacervation in relation to inclusions that occur in the cell vacuoles. The whole question of the relation of catechol to growth and cell physiology has taken on greater significance as a result of recent advances in the physical and the biological sciences.

Orange seedlings, grown for a time in a nutrient solution deficient in zinc, were characterized by hypertrophied root tips (Fig. 1) similar to tomato roots described by Eltinge and Reed (4). An inspection of figure 1 makes evident the connection between the macroscopic and microscopic features of the root. There was a strong tendency for the cortical cells to be enlarged radially in contrast with the lack of growth in the axial direction. The globular coacervates in the cells of these affected roots resembled in all essential ways the vacuolar inclusions that the writers found in orange leaves (12). The contrast between healthy and affected root cells is evident from the photomicrographs shown in figure 2, A and B. The former had grown for several months in a culture containing all the necessary microelements. Its postmeristematic cells were free from coacervates with the exception of a few cells near the surface, which were about to be cast off from the growing expanding root tip. The lower figure represents cells from the comparable region of an orange root tip that had grown in a nutrient solution containing all the necessary microelements, except zinc. The section shows the characteristic enlargement of many of the cells and

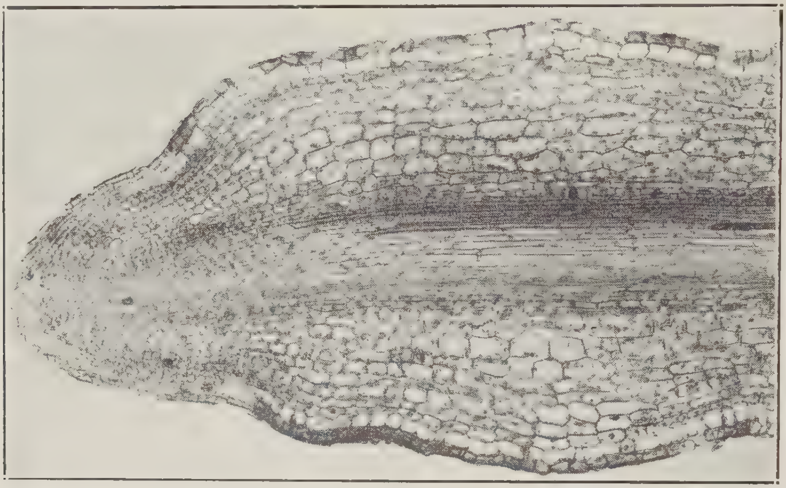

FIG. 1. Longisection of apical region of an orange root grown in a zinc-deficient nutrient solution. The cells of the periblem had been hypertrophied with the production of an abnormal swelling in the post-meristematic region.

the almost universal presence of globular inclusions in the cells. From these the nucleus was easily defined through the use of the molybdenic reagent, which stains the chromocenter of the nucleus a deep blue, as molybdenum blue is being formed at the site of the nucleoproteins.

Figure 3, A, shows in greater detail the contents of healthy cells of the cortical parenchyma of an orange root tip that received all necessary nutrients. The rhomboidal cells shown in this figure contained many short, rodlike mitochondria, arranged along the cytoplasmic strand. The mitochondria had a pronounced tendency to form chains, together with an incipi-

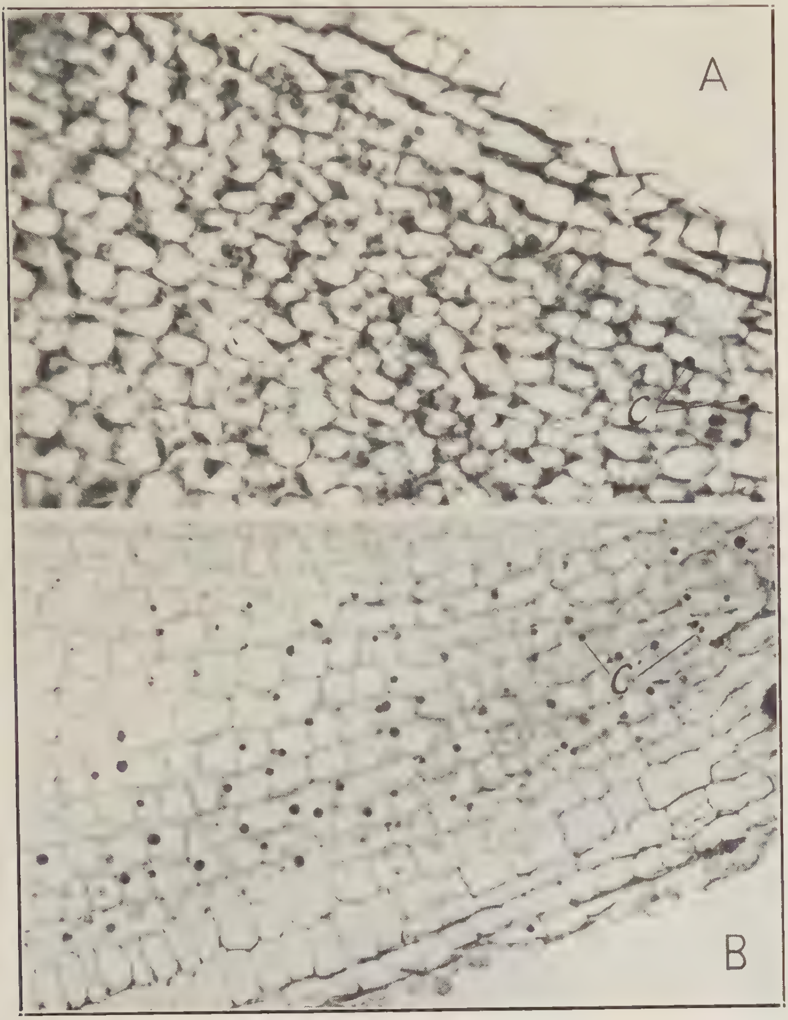

Fig. 2. A. Portion of a longisection of the post-meristematic region of a healthy orange root grown in a complete nutrient solution. The cell vacuoles were free of globular aggregates. B. Portion of a longisection of an orange root-tip that had grown in a zinc-deficient nutrient solution. In contrast to the condition shown in A, the vacuoles of many cells contained globular aggregates (stained with safranin). C, coacervates.

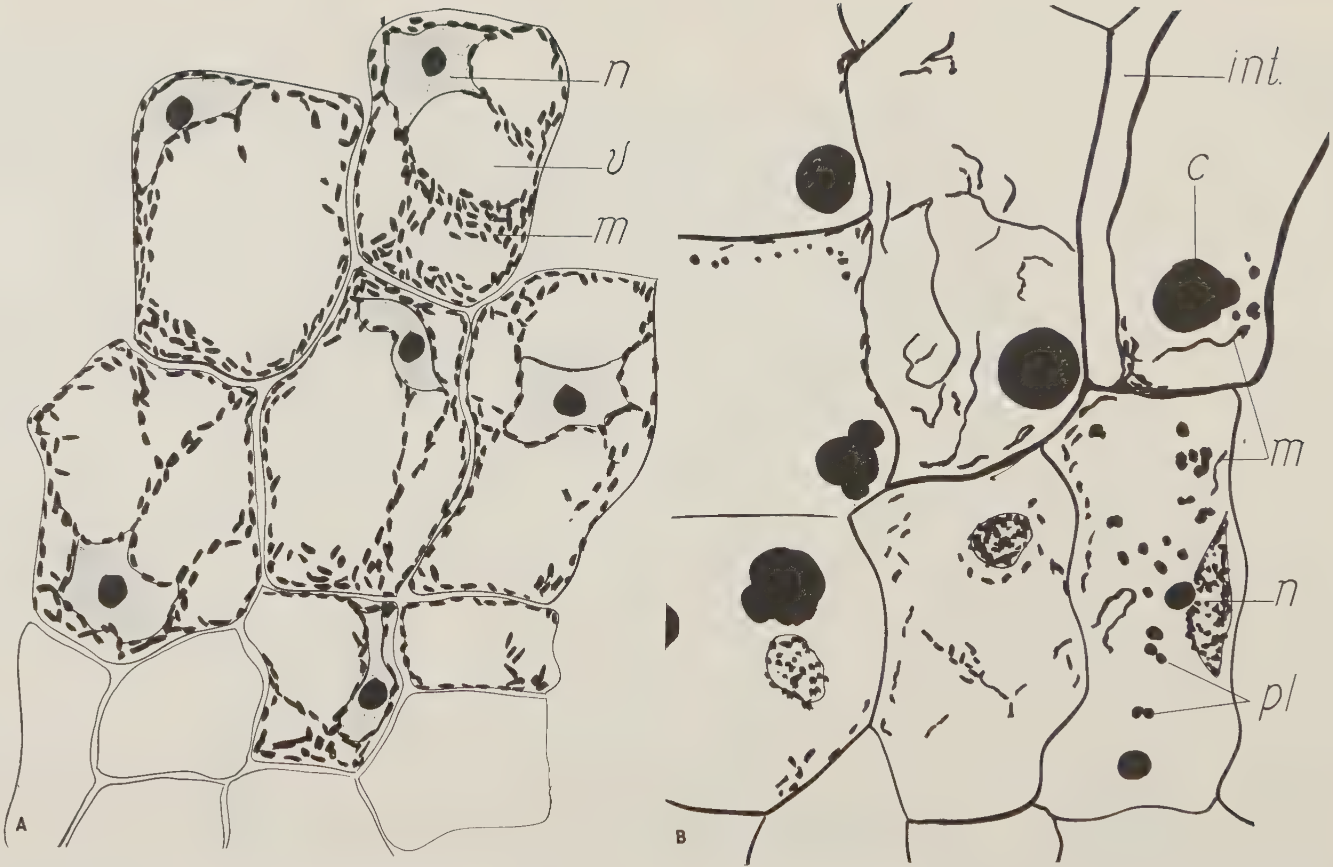

FIG. 3. A. Healthy cells of the cortical parenchyma of orange root grown in a solution containing all the necessary microelements. Vacuolar inclusions were not present. B. Hypoplastic cells from orange roots grown in a solution lacking zinc. In contrast to the healthy cells the vacuoles contained coacervated bodies, and skeinlike mitochondria. The coherence of mitochondria in some of the cells indicates a lowered surface tension. *n*, nucleus; *v*, vacuole; *m*, mitochondria; *pl*, plastids; *c*, coacervates; *int.* intercellular space.

ent development of amyloplasts, with one single starch grain. These amyloplasts were in definite contrast with the huge amyloplasts with numerous starch grains, found in cells of roots deficient in zinc. The nuclei of these cells were large and in many cases were deformed by the pressure of the vacuolar sap.

A striking contrast resulting from the deficiency of zinc in the nutrient solution is shown by cells of an orange root, seen in detail in figure 3, B. The large vacuoles contained conspicuous aggregates consisting of coacervated polyphenol contrasting with the optically empty vacuoles shown in figure 3, A. These coacervates had a tendency to cohere and to produce lobate bodies. It may be recalled that de Jong gave examples of lobate coacervates of casein formed at its isoelectric point, which were characterized by a very low water content.

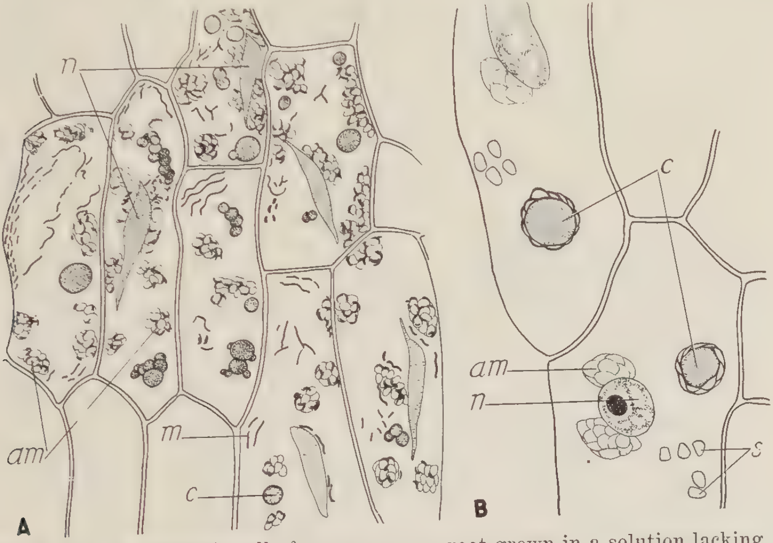

FIG. 4. A. Hypoplastic cells from an orange root grown in a solution lacking all the microelements. The cells were characterized by the large spindle-shape nuclei, by coacervated inclusions, large amyloplasts filled with starch grains, and by filiform mitochondria, stained with acid fuchsin and methyl green. B. Inclusions in cells of an orange root grown without zinc. *am*, amyloplast containing many starch grains; *c*, coacervates showing vacuolate envelope of phospholipoid; *n*, nucleus; *s*, starch.

Stated in its simplest terms it seems that we are observing colloidal bodies that have been separated from the liquid stage by "unmixing" similar to that described in a preceding paragraph. The importance of this simplified drawing will become evident as the discussion proceeds.

The effects of a deficiency of all the supplementary micro-elements on certain cyto-physiological relations of the cell are shown (Fig. 4, A) by cells of an orange root. Cells here represented came from an older part of the root than those in figure 3, B, and contained many typical coacervates. Here again it was evident that the smaller coacervates had a tendency to adhere to the larger coacervates forming characteristic clumps. It should be emphasized that coacervates cohere only with other coacervates and with no other structures found in the cell vacuole. The presence of amyloplasts containing numerous starch grains indicates that the amylolytic agencies were inhibited in these hypoplastic cells; consequently, starch was not used in metabolism. The nuclei were abnormally large and were forced into spindle shapes by the mutual pressure of the cell vacuoles. The enlargement of the nuclei would in itself indicate that the cells were in an unhealthy condition. Coacervates, occurring singly and in clumps, could be seen in most of the postmeristematic cells. Especially conspicuous were the definite bounding membranes, owing to their strong affinity for acid fuchsin. The picture elucidates some of the salient features of the abnormal physiology of the cells suffering from mineral deficiencies and strongly emphasizes the fact now clearly established that under these conditions plastic building materials were not used for the maintenance of the plant, but accumulated rather in the form of conspicuous aggregates in the cell vacuoles.

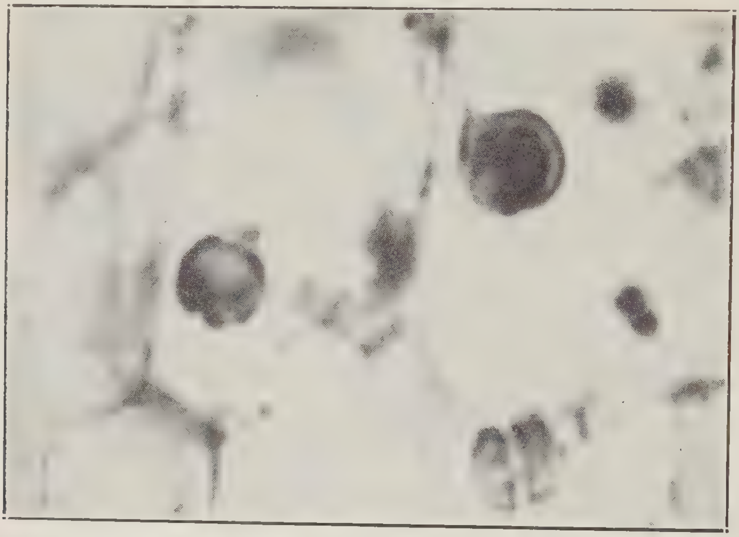

FIG. 5. Coacervated aggregates after treatment with molybdenic reagent, showing the alveolate nature of the surface layers.

Essentially the same cyto-physiological condition was found in other orange root-tips grown in solutions where all of the ordinary nutrient elements were furnished and the only missing microelement was zinc. Figure 4, B shows the large nuclei, the large coacervates with a strong tendency to clump together, and the amyloplasts surcharged with starch, indicating that something had so disarranged their equilibrium that the starch was not used for metabolism. By means of the molybdenum reagent we were able to demonstrate by the characteristic blue color that the delimiting membrane of the coacervate contains phosphoric material. The contrast between the coacervate and the cytoplasm and nucleus was beautifully shown in sections stained with safranin and methyl green, where the coacervates were stained dark-red and the cytoplasm and nuclei a bluish-green.

The nature of the lipoid envelope, which contains water as well as lipoid, may be inferred from the drawings in figure 4, B, and the photomicrograph shown in figure 5. These preparations were obtained by use of the molybdenic acid reagent, which imparts a blue color to compounds containing phosphorus. The amyloplasts also stained blue with this reagent, but more faintly, indicating that they are phosphorylated. In sections correctly handled with the molybdenum reagent, it was possible to see that the surrounding membrane of the coacervate was of an alveolate nature (Fig. 4, B), a fact not demonstrated by the use of such dyes as acid fuchsin. The molybdenum reagent also colored the central mass of the coacervate light-brown, due to the oxidation of the polyphenol to another substance, probably a diketone. In addition to the enveloping membrane, which stained blue with the molybdenic reagent, the figures show that many of the coacervates tend to bulge at the surface. The same thing was seen in sections stained with acid fuchsin, a dye that is also differentially absorbed by phospholipoids.

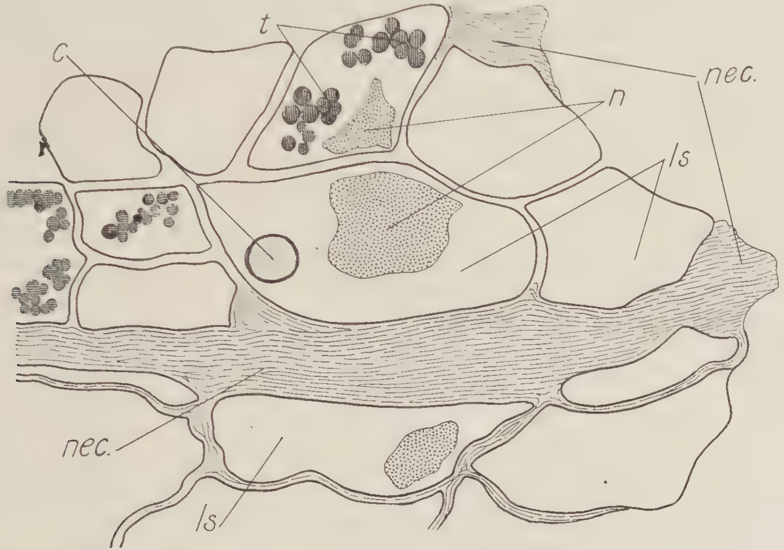

FIG. 6. Cells of a sunflower root showing types of disorganization resulting from boron and zinc deficiency. *c*, coacervate; *t*, globules of phenolic material; *ls*, example of lysis which destroyed the cytoplasm and its derivatives; *nec*, necrotic material; *n*, nucleus.

An inspection of cells like those shown in figures 3, B, and 4, A, strongly suggests that the surface tension of mitochondria and plastids was greatly reduced in the affected cells. The straight or sinuous filaments of mitochondria formed by coherence were characteristic of hypoplastic cells. The adherence of mitochondria to nuclei and plastids illustrated in figure 4, A, is also an indication of the lowered surface tension of the solution. The writers (14) have previously described a characteristic polarization and clumping of the cell contents in affected leaves.

The disturbance in cell physiology caused by a deficiency of boron was revealed by a study of cells in root-tips of sunflowers that had grown for 6 weeks in nutrient solution without any of the accessory substances present. The tops of the plants showed very evident injury from lack of boron and zinc.

Figures 3, B, and 6 show sections of root tips that contain large intercellular spaces, often filled with amorphous material that appeared to be a product of the necrosis of preexisting cell layers. The condition shown in figure 6 indicates that there had been extensive necrosis in the pericycle leaving a band of disintegrated material that absorbed haematoxylin very readily. The adjacent cortical cells that were still living contained globular coacervates or phenolic material. The extent of the injury resulting from the deficiency of boron and zinc is shown also by the lysis that affected the cytoplasm and its derivatives. Eltinge and Reed (4) found similar intercellular spaces in the roots of tomato plants suffering from zinc deficiency, and containing dark-staining amorphous material, which arose from disintegration of cellular material.

We conclude, therefore, from these converging lines of evidence, that we are dealing with a system in which complex coacervates are formed through a change in the equilibrium between the diffused colloidal material and the surrounding liquid. In this relation of molecular orientation the phosphatids probably play a significant role as factors of coacervation because the positively charged choline group is held at the surface of the polyphenol globule and the negatively charged phosphate ions, with adherent hydrocarbon chains, radiate outwardly into the dispersion medium (6).

The coacervated aggregates appear to consist of a central mass of phenols or polyphenols surrounded by a layer of phospho-lipoid material, formed as a result of the disturbance of hydrogen bonds in the catechol-water system by the activity of a catechol oxidase.

The evidence here presented, in addition to the discussion we previously published, Reed and Dufrenoy (12) points the way to a better interpretation of the conditions in the cells of diseased plants. It appears that important processes are intimately dependent upon small amounts of ions, such as zinc and boron, which tend to keep polyphenols and phospholipoids dispersed in the vacuoles of living cells. When they are absent, there may be a lowering of "solvation," resulting in flocculation or in coacervation. The specific activity of these metallic ions remains to be investigated.

SUMMARY

Our previous observations upon globular refringent bodies in the cells of abnormal leaves have been extended now to other parts of plants. These bodies have been found in roots and buds, as well as in leaves, and are interpreted as evidence that there has been a decided shift in the metabolic equilibrium of the cells.

Chemists have shown recently that colloids, which were dispersed in an aqueous medium, may become concentrated into definite, stable globules by a process analogous to flocculation and have designated them as coacervates.

The coacervates herewith described in affected plant cells are considered colloids (mostly made up of catechol compounds) that separated out from the suspending fluid and were, consequently, condensed into spherical bodies floating in the saline solution of the vacuole. Those condensed colloids are surrounded by a precipitation membrane made up of phosphatids, meaning a complex of lipoids and phosphorus.

Those colloids, chiefly at the periphery, at the interphase with the limiting membrane, are the site for high oxidase activity. Therefore, they become the site for the attraction of positively charged ions and, conversely, a center of dispersion for negatively charged ions, such as PO_4. Phosphatids, however, may be considered to be amphoteric, and, therefore, could concentrate at the limiting surface, at the interphase, there to build up a limiting membrane.

Coacervates were found in the vacuoles of cells of roots grown in solutions lacking one or more of the important supplementary micro-elements. These bodies, however, were not found in the cells of roots receiving the necessary micro-elements. They were identified by their form, their stain reactions, and their location in the cells.

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THE TRANSIENT FEEDING OF ROOT–KNOT NEMATODE LARVAE[1]

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Difficulties inherent in the examination of living nematodes in association with their hosts have impeded investigations of how these parasites enter roots and how they induce the local necrosis, hypertrophy, or abnormal tissue differentiation characteristically associated with different root-infesting species. In 1937, the writer (5) described the feeding habits of the root-knot nematode, *Heterodera marioni* (Cornu) Goodey 1932, after it had attained its permanent site, and thereby helped to clarify the problem of how this parasite stimulates the development of giant cells and galls, but he did not determine when feeding begins nor how the nematode makes its way into a root. Certain pathological effects of this nematode are still not well defined, for they are induced before the larva has completed its penetration into and migration within its host to its site of permanent feeding. New refinements of techniques have now permitted observation of larvae before and during their entry into roots, with results that further elucidate the pathogenesis of root knot.

LITERATURE REVIEW

At the time Christie (2)[2] reported his thorough histological study of the development of root-knot nematode galls, the manner in which this parasite feeds was still the subject of hypotheses that Linford (5) later determined, by examination of living material, to be somewhat erroneous. It was found that giant cells develop under the stimulation of a persistently repeated cycle that includes: (a) puncture of the cell wall by the protrusible hollow buccal stylet or spear of the nematode; (b) injection of saliva into the cell; and (c) sucking out of only part of the cell content. Those observations were made on nematodes that had completed their entry into roots and had assumed their fixed positions for feeding. The few inadequate observations of larvae still in migration failed to detect feeding but did demonstrate that the stylet is used in opening a passageway.

The initiation of pathological disturbances prior to the parasite's reaching its permanent feeding site has been indicated by various investigators. Godfrey and Oliveira (3) found that heavy infestations may retard or stop root elongation within 24 hours and cause visible lateral enlargement of the root tip within 48 hours, even though the larvae have not, within that time, reached their feeding sites. They state (p. 329) ". . . the apparent swelling is due to retardation of forward growth of the root and abnormally directed growth of the weakened meristematic tissues rather than to true gall development."

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[1] Published with the approval of the director as Technical Paper No. 136 of the Pineapple Research Institute, University of Hawaii.

[2] See this paper for a comprehensive statement of the development of galls and of the group of highly modified giant cells at the head of the parasite, with a review of earlier literature.

Christie (2) observed that when considerable numbers of larvae enter a root simultaneously, growth may be stopped within 24 hours, and suggested that the presence of the parasite in the meristem suppresses mitotic activity. He also found very prompt reaction of root cells to the presence of the larvae, showing first in hypertrophy of cortical cells ". . . not confined to cells lying adjacent to the path of the larva . . . ," and also sometimes of endodermal and pericyclic cells. Some cells are destroyed and some passed through by the migrating larva, Christie found, constituting exceptions to the general rule that migration is intracellular with little injury to the cells.

Linford (9), studying the development of cowpea shoots from experimentally infested buds, found severe leaf deformities, sometimes out of proportion to numbers of nematodes present, as well as stoppage of terminal buds; and suggested that these disturbances might be results of mechanical injuries inflicted by migration of larvae through meristematic tissues, separating the cells and thereby disturbing the coordination of tissue differentiation.

Godfrey and Oliveira (3) and also Linford (7) have called attention to the phenomenon of mass attack upon root tips, numerous larvae entering the root in closely packed groups, and Linford (9) has demonstrated experimentally the tendency for larvae to enter through wounds where such are available, but these workers have not considered the factor of mechanical injury resulting from such mass attacks in the etiology of nematode galls.

METHODS

Observation of the activities of larvae before and during penetration into roots required the development of a technique that would permit observation at high magnification with both roots and nematodes firmly supported in a moist medium but with the larvae free to move. Details of apparatus and methods are reported elsewhere (10). Briefly, however, roots were allowed to grow against a coverslip in soil or other granular medium, either in a ring chamber formed by cementing a coverslip onto a glass ring, or in a miniature root observation box (8) modified by cementing a coverslip over an opening in one side. For very small seedlings the ring chamber with a thin layer of dark soil or sand was excellent if the chambers were held in a moist chamber between observations. Observation was with the aid of a 40× achromatic water immersion objective and cooled incident light. Somewhat of a dark-field effect was obtained, revealing ample detail to permit identification of the nematode species and observation of its activities. Where the host protoplasm was not too abundant it sometimes was possible to see significant details through 1 or even 2 layers of root cells.

Some of the observations of active migration and of host injuries were made with washed roots and transmitted light. Use also was made of materials processed with either Fleming's fluid and clove oil or lactophenol with cotton (anilin) blue or acid fuchsin. Fixation for paraffin sections was in Karpechenko's fluid.

Heterodera marioni larvae for these studies were hatched from egg masses on cowpea roots, but were of a strain obtained originally from a pineapple field. After hatching in shallow water they were decanted and finally passed through filter paper as described elsewhere (9) to free them from débris.

Observations were made with roots of various plants, although lettuce was employed somewhat more than others. Primary roots of *Portulaca grandiflora* have the advantage of being so slender and translucent that activities of nematodes may be observed dimly almost in the center of the root by either reflected or transmitted light. Such slender roots are less satisfactory for observation of the early stages of penetration because their small radius of curvature leaves only a narrow surface in contact with the glass, and relatively few nematodes penetrate from a position advantageous for observation.

OBSERVATIONS

The Process of Feeding

The feeding process in young, transient *Heterodera marioni* larvae is very similar to that already described (5) for older individuals. The larva punctures a cell with its stylet, then lies at rest briefly before sucking out part of the cell contents.

The puncturing of a cell wall is accomplished only with difficulty despite the extreme slenderness of the stylet tip. A larva thrusts its stylet chiefly when its body is so braced that it may hold its head firmly against a resistant surface; but, when it is favorably situated and is apparently trying to puncture a cell wall, the rapidity and persistency with which it thrusts are remarkable. One larva, observed continuously during 36 minutes, was seen to thrust its stylet at approximately the following rates, expressed as numbers per 30 seconds: 52, while the larva slowly moved its head over two cells; 81, with the head held securely in one place; 58, while again exploring; and 107, immediately before puncturing a cell. By thrusting at such rates and sometimes for many minutes against a small area of a single cell, the nematode gradually weakens the resistance of the wall. Perhaps diffusion of some substance through the injured wall may provide the stimulus for the nematode to persist in its attack in one place and finally to exert the special effort required for puncturing. After a usually prolonged series of thrusts in one place with gradually quickening tempo, the stylet is finally inserted into the cell with a series of three or more very rapid jerks, the stylet not sliding back between thrusts, and each one carrying it farther forward until the maximum possible degree of protrusion is achieved.

Once the stylet tip is fully inserted into a cell, all perceptible motion of the nematode stops. Sometimes, after only a few seconds, the stylet is withdrawn either with or without 2 or 3 preliminary pulsations of the esophageal bulb, and the nematode moves to another cell. Usually, however, after a period of 15 to 30 seconds of rest, the esophageal bulb begins a rapid and rhythmic pulsation that may continue 10 to 40 seconds. In the young larva this bulb is relatively inconspicuous and the valve at its center is so small that the action of the bulb can be recognized only with good definition at high magnification. Conditions of observation amply sufficient to detect activity of the bulb in a half-grown or mature female are wholly inadequate here. Pulsation is very rapid, as indicated by the following approximate numbers of complete pulsations per 30 seconds: 90, 95, 120, 91, 95, 93 and 104. These rates were computed from counts made during the usually shorter periods of pulsation while one larva fed on the surface of a lettuce root. It has not been possible in this study to observe movement of cell contents toward the stylet tip nor into the anterior end of the intestine, but evidence from other studies (6) leaves no doubt that this rhythmic pulsation of the esophageal bulb is the mechanism with which such nematodes injest their food.

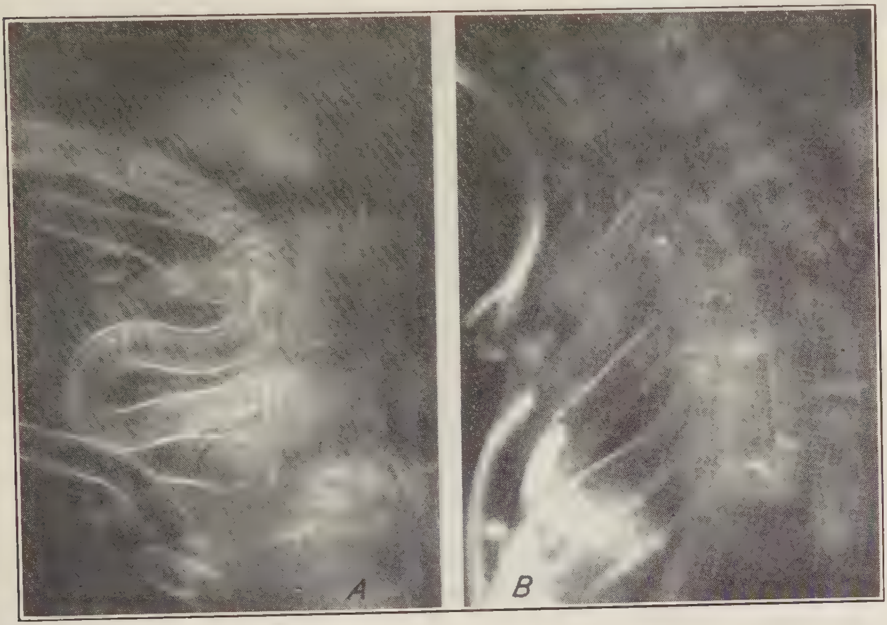

FIG. 1. *Heterodera marioni* larvae associated with lettuce roots, photographed by incident light. × 380. A. Larva in position for initial attempt to feed. B. Group of larvae attacking at a point where injury, begun earlier, has already caused the root to curve.

As soon as action of the bulb stops, the larva retracts its stylet and either immediately resumes thrusting in approximately the same place, turns its head to a different cell, or crawls to a different position before the next period of feeding.

Feeding from Epidermal Cells

A larva may begin thrusting its stylet intermittently soon after it makes contact with the root, even in a relatively mature zone (Fig. 1, A), but successful piercing of a wall has not been seen in epidermal tissues older than where root hairs are beginning to develop. As has been described previously (7), the larva characteristically migrates down the root toward its tip. Some feeding occurs in the old to intermediate elongating zone, but a larva rarely persists in such a place long enough to enter the root. Instead, after feeding in a cell or two, it advances to the young elongating zone or the meristematic region. During such movement it may feed in several cells, and in one cell several times. No instance of feeding in a root hair itself was observed, although several times larvae were seen to feed in the basal portion of a cell from which a root hair was developing. Some epidermal cells look no different under the conditions of observation employed here after having been fed in than do normal cells, but dermatogen cells, after having been fed in several times, frequently appear much disorganized. Time intervals between separate feedings are varied, even when the larva is in a favorable site and is beginning to penetrate, commonly ranging from two to four minutes. Much longer intervals may occur when feeding is interrupted by locomotion.

Feeding During Entry and Migration Within the Root

After a dermatogen or young epidermal cell has been fed in one or more times and battered by innumerable stylet thrusts during a period of minutes, its wall may break, allowing the head of the nematode to slip in while the disorganized remains of the protoplast flow out into the water film on the root surface. The larva then commonly continues to penetrate into the root, feeding as it goes. Bacteria and protozoa congregate at such points and may enter the wound. Other larvae also may be attracted to this point (Fig. 1, B), where they attempt to invade the root together. With the still heavier infestations illustrated by Godfrey and Oliveira (3) and by Linford (7), the larvae crowd so closely together that directional movement of the individual is limited. Within such masses they may lie almost motionless but continue thrusting their stylets and breaking down numerous cells. Apparently, they disturb one another to such an extent that they less frequently are able to feed than when distributed singly, and certainly larvae in groups are less favorable for observation of the details of penetration.

After an individual has forced its head into a cell, its feeding continues much as on the root surface, periods of rapid thrusting of the stylet alternating with periods of rest and of sucking. It uses its stylet also both to break through walls and to separate cells along the middle lamella. A larva may break into subepidermal cells—masses of larvae characteristically do—but generally, as reported by other investigators, the individual larva pushes between cells as it moves farther into the root. Sometimes, where a subepidermal cell has been entered, it has been possible to observe the thrusting of the stylet into the second layer of cortical cells, with activities similar to those on the root surface. Even when the head of the nematode could no longer be seen, the occasional period of pulsation of the esophageal bulb gave evidence that the same mechanism of feeding was persisting.

A few fortunate observations have been made on larvae that were migrating in a subepidermal position, crowding between cells of the first subepidermal layer, so that feeding and migration could still be observed after ⅔ of the body length was within the root. Such larvae fed repeatedly from cells on either side of the head, and also thrust the stylet directly between cells. These larvae were in young tissues where cell walls appeared to afford little resistance, and the intervals between feedings were shorter and more regular than on the root surface or in a more mature part of the root. As on the root surface, one cell frequently was fed in more than once. These larvae, in agreement with numerous others that have been observed after penetration was almost complete, entered the root with a halting motion: even when only a little of the tail was exposed to view, the nematode was seen to advance approximately one cell length or less, and then lie at rest for 2 minutes or more before moving farther in. Such halting advance has been observed with larvae penetrating almost directly into the meristem through the side of the root cap and with others entering the maturing zone. Consequently, there is reason to think that intermittent feeding characteristically continues throughout the period of migration. Exceptions have been noted, such as larvae observed within the stele of *Portulaca grandiflora* roots where they were completely surrounded by well differentiated tissues (Fig. 2, A). Such

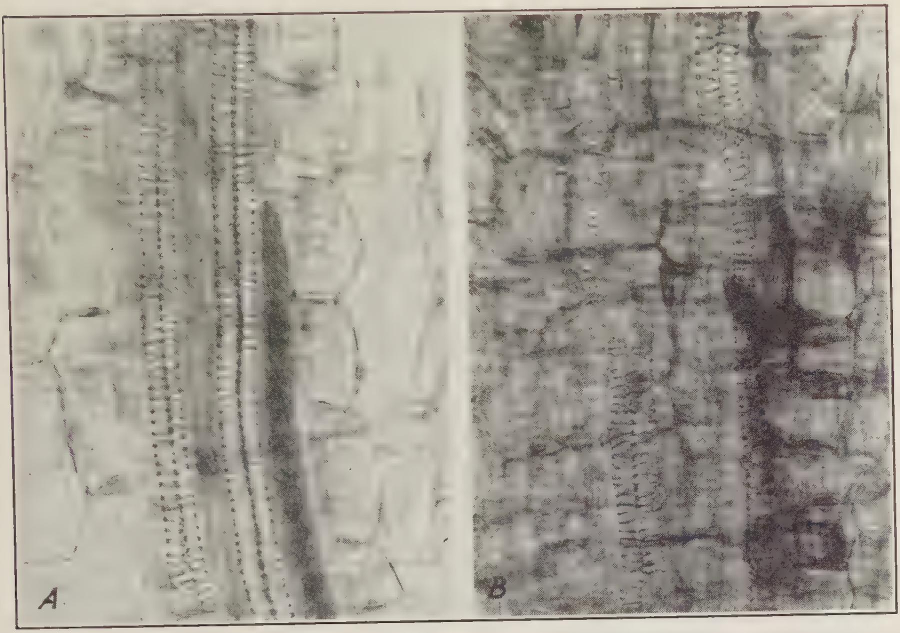

Fig. 2. *Portulaca grandiflora* root processed with lactophenol and anilin blue 2 days after infestation with *Heterodera marioni* larvae. The root tip was distinctly swollen, but the only 3 larvae present were in the mature stele, migrating up the root. ×380. A. Larva between endodermis and xylem, apparently wedged between pericycle cells. Two other larvae, not shown, were between the primary xylem bundles. B. Part of the swollen apical region, showing wide separation of xylem bundles and interrupted xylem elements. The dark areas at right of center were brown, apparently marking the path of entry of one or more larvae.

larvae, observed in the living condition before the root was processed, appeared to be trapped in spaces so narrow that they were unable to turn about, and between walls too heavy for them either to break down or puncture for feeding.

Entering larvae very commonly are found headed toward the root tip, perhaps in consequence both of having migrated down along the root surface

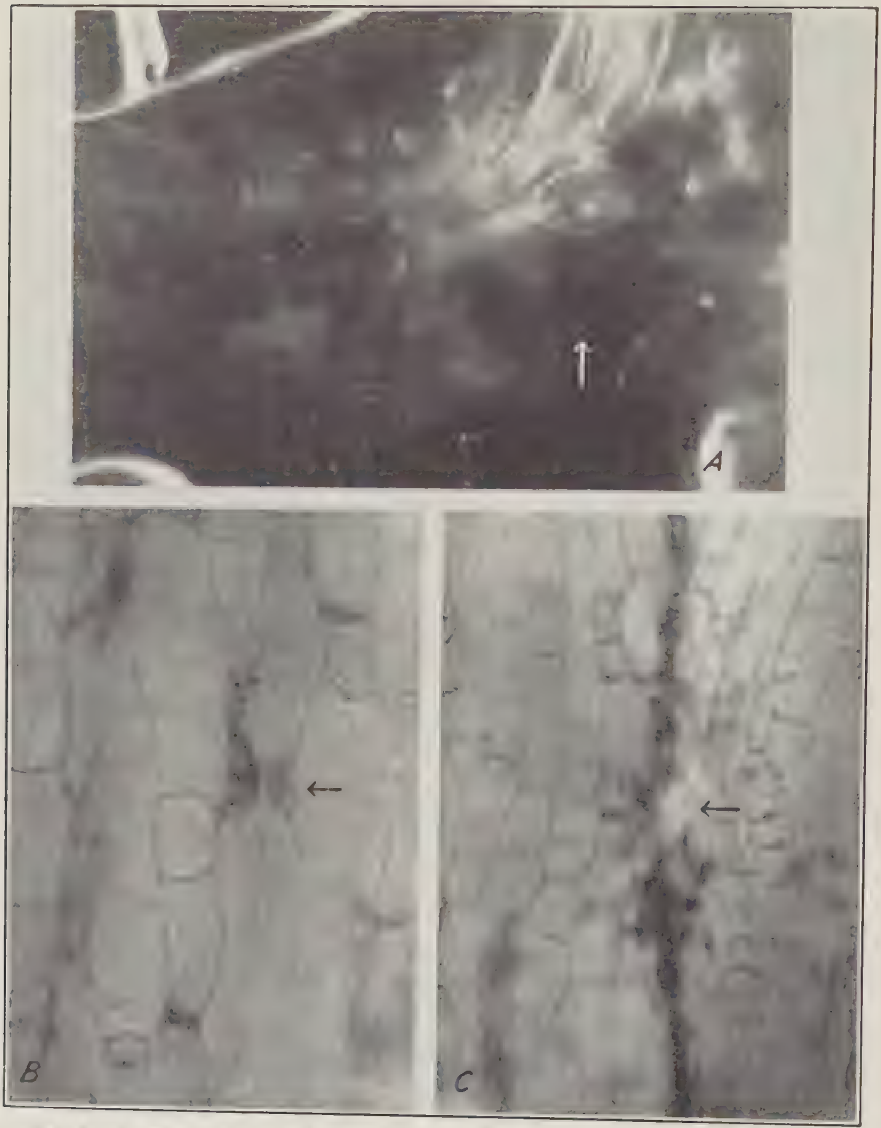

FIG. 3. A. Several larvae (upper right) entering a Portulaca root at the edge of a deep wound (at arrow) begun by earlier penetrants and enlarged by growth of less injured tissues. Photographed by incident light. × 380. B. Disturbed cell pattern in young epidermis of lettuce root infested 50 hours earlier with *H. marioni* larvae. The arrow marks a hole left by incomplete closure of a destroyed cell. × 380. C. Like B, but with a conspicuous line of injury and with a deep hole at arrow. × 190.

prior to penetration, and of the forward motion of the root tip through the surrounding medium during early stages of entry. Many such larvae, in slender roots, continue in the same general direction to the terminal meristem where the altered orientation of cells or contact with less nutritious root cap cells cause them to turn about and start back up the root.

Some Pathological Effects

As reported by Christie (2) and earlier workers, larvae move chiefly between cells without destroying them. In paraffin sections, however, the writer finds a tendency for cells just behind the head of a migrating nematode to show compact nuclei and sometimes a mild shrinkage of the entire cell. These changes may represent abnormal responses to the fixing fluid, but probably the basic cause is the altered cell constitution resulting from feeding. No attempt has been made in this study to follow cytological changes in these cells, but several slender roots have been seen showing hypertrophy where larvae have not established for permanent feeding. The *Portulaca grandiflora* root shown in figure 2, A, B, contained 3 larvae, all in the stele of the mature zone. The root tip was conspicuously swollen, and the stele itself was hypertrophied, evidently in consequence of earlier feeding of these larvae during migration through the meristem.

Cells destroyed by the entering larvae are much more numerous in the dermatogen and outer periblem than farther in. Distributed singly or in small groups, such broken cells tend to be crushed closed by enlargement of adjacent tissues, leaving only minor irregularities in root shape beyond the disturbed cell pattern (Fig. 3, B, C). More extensive injuries, resulting from the attack of numerous larvae on a small root, sometimes result in deep holes (Fig. 3, A) or in cracks that may extend into the differentiating stele. Such cracking probably is attributable in part to hypertrophy such as Christie (2) found to begin promptly after larvae enter a root, but here occurring more abundantly and under a broken epidermis.

In the most extreme instances, the entire root tip is disintegrated without gall formation. This has been observed with roots of various plants attacked by numbers of larvae comparable to those shown in figure 1 of a preceding paper (7). In none of these observations have bacteria been excluded, but from the promptness of disintegration when well washed larvae were added to essentially aseptic seedling cultures, as well as from the actual observation of tissue destruction by larvae, there is little doubt that the nematodes themselves are basically the cause of such disintegration of root tips.

DISCUSSION

The rhythmic processes of feeding shown by young *Heterodera marioni* larvae on the root surface and during entry into the root are essentially similar to those described earlier for large females, the chief differences being in rates. Not only does the larva rest more briefly after inserting its stylet into a cell, and then feed for a shorter time, but also its stylet thrusts and the pulsation of its bulb are more rapid. No evidence was obtained that the young larva injects saliva into the cell before sucking out cell contents, but the period of rest prior to pulsation appears analogous with the periods of demonstrated saliva flow in predacious species of *Aphelenchoides* (6) and in *Heterodera marioni* females (5). The essentially non-lethal effects of the feeding of these larvae is attested by the scarcity of dead cells along paths of migration. The hypertrophy of some such cells described by Christie (2), the false gall formation reported by Godfrey and Oliveira (3), and the distortion of some lightly infested aerial parts reported by Linford (9) apparently are all related to the feeding of migrating larvae.

The factors that cause a larva to terminate its migration present an interesting question. Mere selection of cells in which it is able to feed does not answer, otherwise larvae would not have left the meristematic region and migrated back into the mature zone, as shown in figure 2, A, B. This condition has been seen only in very slender roots, suggesting that migration stops only after the larva has already fed during some moderate period. These observations also raise, but do not answer, the question of whether a larva too nearly starved to be able to enter a root may, by feeding on the root surface, so replenish its reserves that it can later expend the energy required to force entry.

Such physical injuries to root tips as are here described probably are negligible at the beginning of the crop season under normal conditions. After hatching of first or second generation eggs has begun, however, especially from exposed egg masses, local concentrations of larvae undoubtedly become sufficient to terminate growth of new rootlets in close proximity to such egg masses. Many instances of rootlet disintegration formerly regarded by the writer as results of mildly pathogenic microorganisms invading roots of plants weakened by root knot, now appear primarily attributable to the root-knot nematode itself.

The observed entry of microorganisms from the soil into superficial root cells destroyed by the penetrating larva raises the question of why bacteria are not conspicuously associated with the enlarging or mature nematode at its permanent feeding site. Ark and Thomas (1) and Kalinenko (4) have demonstrated that bacteria commonly accompany certain nematodes that, however, enter chiefly into maturing tissues rather than near the apical meristem. Perhaps both the gradual posterior taper of the *Heterodera marioni* larva to a relatively slender terminus, and the vitality of meristematic and enlarging root cells, result in too prompt and complete closure of the migration path for bacteria to be carried far in appreciable numbers. Or possibly bacteria do play a rôle in gall formation that has not yet been recognized.

The ability of *Heterodera* larvae to both macerate root tips and cause cracks that extend into a differentiating stele may be as significant as the early disintegration of galls in the observed tendency for heavy root-knot infestations to accentuate Fusarium wilt of cotton, even in wilt-resistant varieties.

SUMMARY

The feeding of *Heterodera marioni* larvae was observed at 600 diameters magnification with cool, incident light and with roots growing in soil or other granular medium against coverslips.

Larvae may feed repeatedly from epidermal cells before entering the root. They continue feeding as they enter into and migrate within it.

The stylet tip is inserted into a cell after rapid and persistent thrusting against the wall; the larva then lies apparently at rest 15 to 30 seconds (analogous with the period of saliva flow), next sucks 10 to 40 seconds with its esophageal bulb pulsating vigorously, and finally retracts its stylet.

Young epidermal or dermatogen cells, especially at the point of entry of the nematode, are killed not by feeding but by entry of the larva after the wall has been weakened by the stylet. Individual larvae similarly kill some cells within the root.

Larvae attacking in groups destroy many cells, causing holes, cracks into the young stele, or, with extremely heavy infestations, disintegration of root tips.

Feeding during entry and migration appears to explain cessation of elongation and the swelling of root tips while larvae are migrating, as well as the early hypertrophy of cells remote from the permanent feeding site.

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STUDIES ON BLACK STEM OF ALFALFA CAUSED BY ASCOCHYTA IMPERFECTA[1]

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INTRODUCTION

A black-stem disease of alfalfa in Kansas, the cause of which had not previously been published, has been observed for many years. It occasionally produces serious defoliation and death of the stems during cool wet spring seasons.

Although black stem is of less economic importance in the dry climate of Kansas, seed from this State often is used in humid regions where this disease is more prevalent. This paper deals with a study of the characteristics of the disease, the identification of the causal organism and the methods used in artificial inoculations suitable for a systematic testing of alfalfa breeding material. Development of black-stem resistant selections of alfalfa offers one method of control.

REVIEW OF LITERATURE

Stewart, French, and Wilson (8) of New York described black stem of alfalfa in 1908, and attributed it to an undescribed species of *Ascochyta.* The fungus was named *Ascochyta imperfecta* in 1911 by C. H. Peck (3).

Valleau and Fergus (10) in 1929 reported a similar disease of unknown origin on alfalfa, sweet clover, and red clover, in Kentucky, which they referred to as black stem. In 1933, Johnson and Valleau (2) found a different organism on each of these legumes. The disease of alfalfa was attributed to *Phoma medicaginis* Malbr. and Roum. A black-stem disease of alfalfa was reported in Idaho in 1936 by Remsberg and Hungerford (4), which was considered the same as that reported by Valleau and Fergus, and Johnson and Valleau, in Kentucky. Toovey, Waterston, and Brooks (9) of England compared cultures of *Phoma medicaginis* Malbr. and Roum., received from F. R. Jones of Wisconsin and E. M. Johnson of Kentucky, and *Ascochyta imperfecta* Peck, received from Jones and R. Sprague, with the British fungus causing black stem, and concluded that all of these cultures were the same species, namely, *Ascochyta imperfecta* Peck. In 1934, Richards (5) of Utah reported a stem blight on alfalfa of undetermined origin which closely resembled the disease caused by *A. imperfecta.* Similar diseases of alfalfa are attributed to *Ascochyta medicaginis* Fckl. by Rosella (6) in France, and *Phyllosticta medicaginis* (Fckl.) Sacc. by Corneli (1) in Italy.

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[1] Contribution from the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, cooperating with the Department of Botany, contribution No. 420 and the Department of Agronomy, contribution No. 327, Kansas Agricultural Experiment Station, Manhattan, Kansas.

[2] Junior Agronomist, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Head, Department of Botany, Kansas Agricultural Experiment Station, respectively.

[3] The authors gratefully acknowledge the assistance and advice of Dr. D. B. Creager, C. O. Grandfield, and C. O. Johnston during the course of these studies.

There is a lack of agreement on the cause of black stem of alfalfa, and some of the conclusions reached in the literature are indefinite. In view of this fact the writers believed it essential to determine the cause of this disease as it occurs in Kansas, before attempts were made to study other phases of the problem.

THE DISEASE

Economic Importance and Geographic Distribution

Detailed observations of black stem were made in the alfalfa plots of the Kansas Agricultural Experiment Station at Manhattan, in 1939. Although infection was light compared with that of previous seasons, a loss of over 15 per cent of the leaves occurred on some of the plots. This loss is of importance when it is considered that the leaves contain a much higher percentage of protein and minerals than the stems. In seasons of severe infection many shoots are entirely killed.

Johnson and Valleau (2) reported that during long, wet spring seasons black-stem kills the early crop of shoots, forcing new crown buds to push out from below the soil surface. On poor soil during favorable black-stem seasons infection was severe enough to destroy stands. Richards (5) reported a loss of 40 to 50 per cent of the yield during a severe outbreak in Utah.

The disease has been found in many parts of Kansas and Nebraska. It also has been reported in New York (8), Kentucky (2, 10), Utah (5), and Idaho (4). In England (9) it was reported in Hertfordshire, Suffolk, Norfolk, and Bedfordshire and it may have been this same disease which was reported in France (6) and Italy (1).

SIGNS AND SYMPTOMS

The earliest symptoms in spring are small, dark-brown or black spots on the stems and leaves. Young stem lesions often are surrounded by water-soaked areas that become slightly raised in later stages of growth. Severely infected stems turn dark brown or black and die (Fig. 1, A). Immature pycnidia develop in the older stem lesions in the winter. By the following spring the stems are covered with numerous black pycnidia (Fig. 1, D).

Under cool humid conditions, the leaf lesions enlarge rapidly, coalesce, and vary in size and shape. They are occasionally zoned and surrounded by chlorotic areas. Petiole lesions occur as numerous, small, black or brown spots, or elongated, diseased areas, which partly or entirely girdle the petiole causing the leaflet to wither. Stipules may become diseased (Fig. 1, C). Diseased leaves turn yellow and wither before they drop. Such leaves usually contain obscure, immature pycnidia. Leaf shedding commences at the bottom of the stem and progresses upwards as the disease becomes more severe (Fig. 1, B).

Field Observations

The extent of the disease in a field is directly correlated with temperature and humidity. It is most severe on the first crop of alfalfa, which usually is produced during the coolest and wettest period of the growing season. In Kansas the infection is negligible on the second and third cuttings, but increases considerably on the fourth crop, when the weather is again cooler and more humid. Small lesions may be observed on leaves and stems in 3 or 4 days following a rain. Prolonged cloudy, rainy weather is favorable for numerous infection periods and rapid spread and development of the disease.

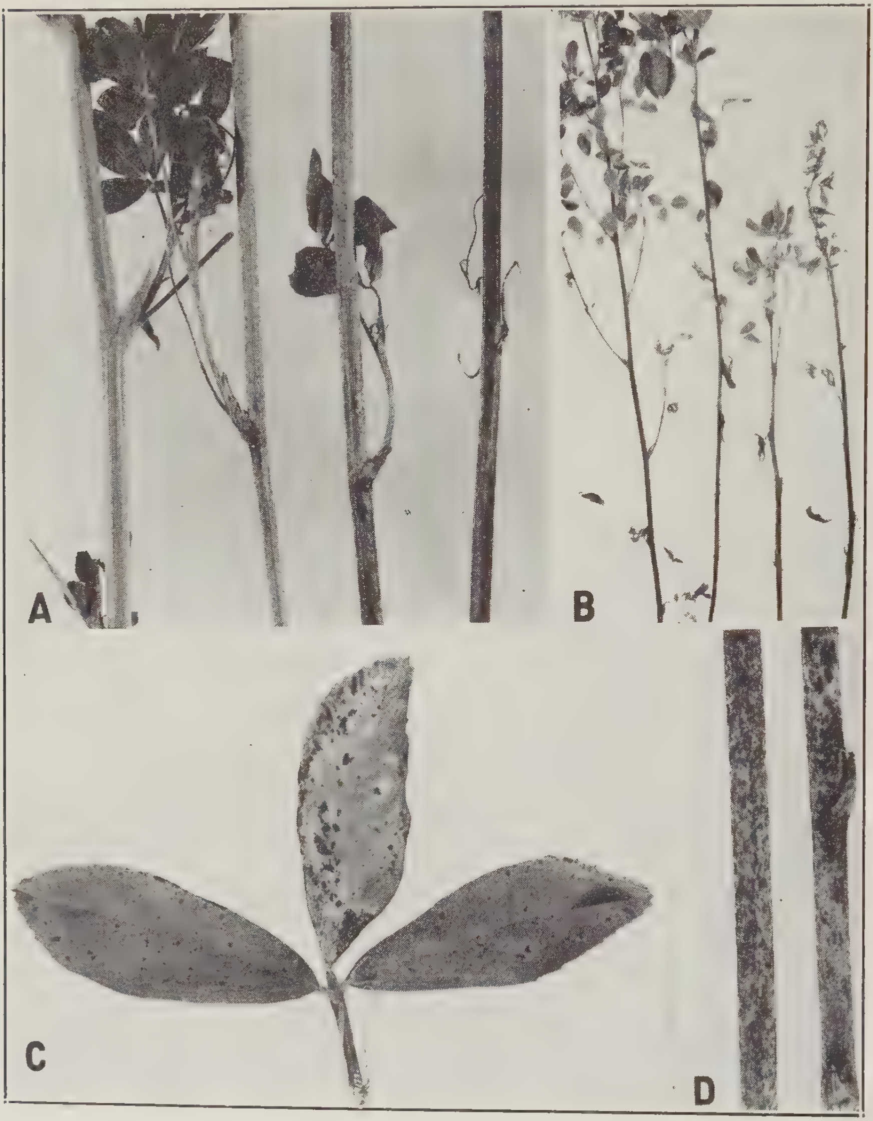

FIG. 1. A. Progressive stages (left to right) in the development of black stem on alfalfa stems. B. Progressive stages of defoliation (left to right) of alfalfa resulting from increased severity of infection by black-stem; C. Black-stem lesions on the petiole, stipules, and leaves of alfalfa; D. Pycnidia of *Ascochyta imperfecta* on overwintered stems of alfalfa.

THE CAUSAL ORGANISM

The causal organism of black-stem of alfalfa in Kansas was identified as *Ascochyta imperfecta* Peck. On potato-dextrose agar, the mycelium was greyish, olive-green, with a whitish margin, and produced abundant pycnidia containing both uniseptate and unicellular spores of variable size, but of dimensions within the range of 6–15 × 2.5–4 μ as first described by Peck (3) and later by Toovey, *et al.* (9).

Life History

Infection takes place early in the spring when the first shoots of alfalfa appear. Spores are disseminated by rain drops that strike diseased over-wintered leaves and stems (Fig. 1, D). The spores germinate within a few hours and the germ tubes enter the alfalfa leaves and stems, probably by direct penetration. The mycelium grows rapidly through the tissue, and diseased areas may be seen with the naked eye 3 to 4 days after infection has taken place. Infected tissue becomes necrotic and pycnidia soon develop.

The organism remains dormant in the host tissue during the dry, hot season, but fruiting is resumed in the fall, with the coming of cool, humid conditions. The inoculum for the fourth cutting was found originating chiefly from diseased crop residue from the first cutting. Spores are expelled in a continuous stream through the ostiole of moistened pycnidia, following rains. Diseased crop residue from the fourth cutting is the most prolific source of inoculum for spring infection, although spores remain viable in diseased stems and leaves from other cuttings. Maturation of pycnidia takes place in early winter, when temperature and moisture conditions are favorable, but probably both the mycelium and pycnidia remain dormant during the winter months.

Temperature Relations

Temperature relations of *Ascochyta imperfecta* were determined by measuring the mycelial growth on plates of potato-dextrose agar at 3-degree intervals, using a range from 9° to 33° C.

Spores originating from single hyphal-tip cultures were allowed to germinate on the agar before the plates were transferred to the incubators. Growth measurements were made 4 days after exposing 2 series of 45 plates each to 9 different temperatures.

Growth was very slow at 9°, but increased up to 21° and fell off slightly at 24°. Above 24° growth decreased rapidly; very little occurred at 33° (Fig. 2).

The margins of colonies were of a frayed and cottony appearance in the 9° to 18° temperatures, but became clearly defined at the higher temperatures. The cottony margins of the colonies where no fruiting occurred became narrower with increasing temperatures. Pycnidia were produced at the periphery of the colonies at 27° and 30°, but none occurred at 33°. Fruiting took place from 9° to 30°. Pycnidia, produced at lower temperatures, were small and translucent but became darker at higher temperatures. The cultures kept at the 18° to 24° temperatures were olive green. The 27° cultures were darker colored, while the 30° and 33° cultures were dark- and light-tan, respectively.

Further observations of the effect of temperature on fruiting were made on sterile alfalfa leaves. Leaves of uniform size and age were selected from a single plant and placed on microscope slides. These were placed in small moist chambers. Three leaves were placed on each of 27 slides in a like number of moist chambers and autoclaved at 15 lb. for 20 min. After cooling, a suspension of spores from a pure culture was placed on each leaf, and 36 hours were allowed for germination before placing the leaves in the incubators. After 6 days the leaves were carefully examined.

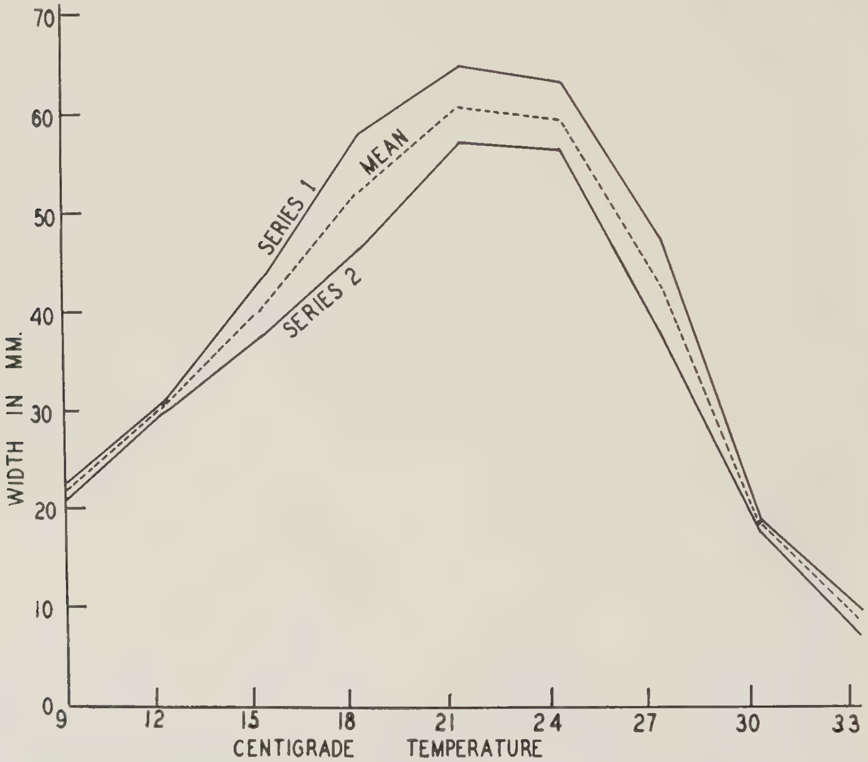

FIG. 2. Temperature relations of *Ascochyta imperfecta* determined by measuring the width of colony at the end of four days.

Pycnidia were produced throughout the range of temperatures, but were exceptionally abundant at 21° to 27°. They were larger at 21° and 24° than at any other temperature, but most numerous at 27°. Approximately 1,500 pycnidia were counted on one leaf at the 27° temperature. These pycnidia produced no spores during the experiment.

Inoculation Studies

The fungus was grown on potato-dextrose agar, alfalfa stems, alfalfa leaves, and sweet-clover stems in an effort to determine the most satisfactory medium for the production of a large supply of viable spores for artificial inoculations. The best medium proved to be sterile, second-year sweet-clover stems. They were kept moist with an inch of water agar in the bottom of the test tube. Sweet-clover stems inoculated with spores along one side developed cottony mycelium, with pycnidia appearing later (Fig. 3). Spores from cultures prepared in this manner were viable after storage in a refrigerator at about 40° F. for 6 months.

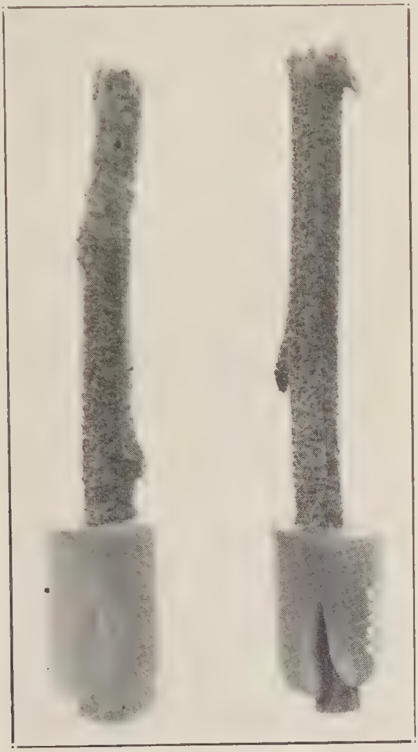

Fig. 3. Pycnidia of *Ascochyta imperfecta* on sterile sweet clover stems.

Inoculations on alfalfa were made by spraying spore suspensions in distilled water on healthy plants that were then placed in a moist chamber for 3 to 5 days, depending upon the season of the year and greenhouse temperatures. A shorter time was required in winter. The relative humidity in the damp chamber was between 90 and 100 per cent, and the temperature between 65° and 70° F., except in summer, when it was higher. Typical young black-stem lesions developed on the leaves, petioles, and stems of alfalfa within 3 days. Stem lesions, however, were less abundant than in the field. On several occasions the fungus was reisolated from inoculated plants and compared with the inoculum, thereby proving its identity and pathogenicity.

The effect of age of the leaf upon ease of infection by the black-stem organism was determined by charting the growth of 3 plants each of Turkestan and Ladak varieties that had previously been cut back to the ground. The purpose of conducting this experiment was to determine if the recovery of different plants or varieties following cutting was a factor affecting susceptibility. The plants were artificially inoculated after charting growth for 23 days. When sufficient time had elapsed for infection, each leaf was scored from 1 to 5, depending upon severity of infection, using as a guide a photograph of diseased leaves illustrating the severity of disease on leaves representative of each number. Consideration was given to size and abundance of lesions. The disease score of leaves of an age group was determined by averaging the score of all leaves within the group. The results are shown in table 1. There is no relationship between leaf age and severity of infection, as determined by this experiment.

TABLE 1

Numerical disease score of leaves of various ages on six alfalfa plants

Plant No.	Age of leaves in days									
	0–4		5–9		10–14		15–19		20–23	
	No. of leaves	Disease	No. of leaves	Disease	No. of leaves	Disease	No. of leaves	Disease	No. of leaves	Disease
Turk. 1	7	2.14	7	2.29	12	2.00	11	2.00	9	2.11
“ 2	12	5.00	16	4.81	16	4.69	22	5.00	2	5.00
“ 3	16	4.69	13	4.69	18	4.61	24	4.88	0	....
Ladak 1	9	3.88	8	4.13	12	3.08	11	2.82	2	2.50
“ 2	9	3.22	9	2.88	12	2.42	15	4.33	0	....
“ 3	10	2.70	10	2.30	12	2.50	8	3.13	0	....
Av.	10.5	3.61	10.5	3.52	13.7	3.22	15.2	3.69	4.3	3.20

HOST RANGE

Toovey, Waterston, and Brooks (9) secured slight spotting of leaves on *Vicia sativa* L. and lesions similar to those on alfalfa on *Trifolium pratense* L., and *Medicago lupulina* L. by artificial inoculation with *Ascochyta imperfecta*. Sprague (7) secured infection to a slight degree on *Melilotus indica* (L.) All., *M. officinalis* (L.) Lam., *Trifolium hybridum* L., and *T. pratense* L. Johnson and Valleau (2) report that *Phoma medicaginis* produced blackening of the stems of red clover and sweet clover, as well as those of alfalfa. No other studies seem to have been made on this phase of the work.

The investigation herein reported served to test the susceptibility of varieties and selections in the field and greenhouse. In connection with the variety test, approximately 50 plants each of *Medicago falcata* L. and *M. ruthenica* Trautv. were found to produce typical lesions when artificially inoculated with *Ascochyta imperfecta*. These species, however, were more resistant to black stem than was common alfalfa. Certain varieties and plants within a variety showed definite indications of resistance. In some cases resistance appeared to be associated with glossy leaves that possibly shed moisture.

Studies on varietal resistance, and other methods of control of alfalfa black stem will be reported in a forthcoming paper.

SUMMARY

Black stem of alfalfa, occurring in Kansas, has been traced to *Ascochyta imperfecta* Peck. This disease causes destructive defoliation and discoloration of the hay crop. It is most severe during cool moist weather, and the first crop of hay is more heavily infected than subsequent cuttings.

The organism probably overwinters as dormant mycelium and pycnidia in crop residue. Field infection takes place by splattering raindrops, which carry spores from diseased tissue to the growing alfalfa shoots.

The optimum temperature for growth of the pathogen in culture was 21° C. Slight growth was observed at 9°, and very little occurred at 33°. Pycnidia were produced throughout the range from 9° to 30°, and were most abundant at 27°.

The most satisfactory method of producing viable spores for artificial inoculations was to grow the fungus on sterile sweet-clover stems in test tubes. Inoculations were made by spraying spore suspensions on healthy plants and placing them in moist chambers from 3 to 5 days. No relationship existed between age of alfalfa leaves and severity of infection following artificial inoculation.

Medicago falcata L. and *M. ruthenica* Trautv. were added to the host range reported in literature.

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LIPOLYTIC ACTIVITY OF PHYTOPATHOGENIC BACTERIA DETERMINED BY MEANS OF SPIRIT BLUE AGAR AND ITS TAXONOMIC SIGNIFICANCE

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The action of phytopathogenic bacteria on fats has received scant attention from students of this group of microorganisms. Jodidi (14) demonstrated crystals of the higher fatty acids in a skim-milk culture of *Bacterium pruni* and accepted this as evidence that this species produces lipolytic enzymes. Castell (5), by means of a modification of Turner's (18) Nile-blue medium, and Castell and Garrard (6), by means of a titrametric technique, showed that *Phytomonas campestris* could decompose fats and that *Erwinia carotovora* lacked this ability. No other mention of lipolytic activity in cultures of related plant pathogens has been found despite a thorough search of the available literature.

Probably one of the reasons why the ability to decompose fats has been overlooked, in studying the physiology of these bacteria, is that methods for detection of this character had not been sufficiently developed to be readily applicable to routine use. It seemed possible that the spirit-blue-agar technique, recently developed by the senior writer (17), might prove satisfactory for this purpose. This medium is essentially a tryptone-yeast extract agar containing a finely dispersed cottonseed-oil emulsion and the indicator dye, spirit blue. It is prepared in sterilized Petri plates for streaking. The fresh sterile medium is pale lavender, if the pH be properly adjusted. Lipolytic[4] bacteria, streaked on the agar, cause the development of a *permanent*, characteristic, deep-blue color beneath and around the colony; colonies of non-lipolytic[4] bacteria produce no comparable change.

It is the writers' purpose to report here on the decomposition of cottonseed oil by various phytopathogenic bacteria as shown by the spirit-blue-agar method, and to evaluate this ability as a taxonomic character.

EXPERIMENTAL

Cultures of the organisms listed in the tables were streaked on plates of spirit-blue cottonseed-oil agar (17). Control plates lacking the cottonseed oil usually were inoculated at the same time, with the intention of guarding against the possibility of development of the blue reaction in the absence of fat. No control plate of this type ever showed a spurious reaction. As an additional precaution, each new batch of the medium was tested with a series of bacteria of known fat-decomposing ability. All cultures were incubated at 27–30° C. and observed periodically for not less than two weeks. Each culture was studied in this manner at least twice and each of the reactions given in the tables is thus the resultant of numerous independent observations.

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[3] The writers wish to express their appreciation to those workers, who by their generous contributions of cultures, have furthered this investigation. An extension of this work is in progress and receipt of any authentic cultures of plant pathogenic bacteria will be welcomed.

[4] The actual reaction shown by spirit-blue agar is possibly more than hydrolysis of the fat. In this paper, the writers wish the term *lipolysis* to indicate that particular action on cottonseed oil that is revealed by the spirit-blue-agar technique, without committing themselves as to the actual mechanism of that action.

In the following tables, the species of the genus *Phytomonas* Bergey *et al.* are allocated to the genera or groups that have been suggested (1, 2, 3, 10, 13) as indicating more correctly the true relationships of these organisms; the reasons for accepting these groupings are discussed more fully below. This division should occasion little inconvenience, since it is essentially the arrangement of the species in the fifth edition of Bergey's *Manual* (2).

DISCUSSION

Examination of these data reveals that the members of the genus *Phytomonas* Bergey *et al.* vary in their ability to decompose cottonseed oil, as shown by the spirit-blue-agar method. This might be expected, since the genus was erected mainly on the basis of plant pathogenicity and, actually, as various investigators (1, 3, 9, 10, 13) have shown, consists of several physiologically unlike groups. In the discussions that follow, it will be shown that the members of any one of these groups usually have a similar and characteristic reaction on spirit-blue agar.

The possible value of lipolysis as a taxonomic character may be indicated by the fact that, regardless of source or age of culture, isolates of any one species give identical reactions by the spirit-blue-agar method. For example, none of the 11 isolates of *Pseudomonas syringae*, received from different laboratories under 9 synonyms, were lipolytic by this method. A similar situation obtained with *Xanthomonas phaseoli*, in which case all the isolates, including two varieties, were lipolytic, even though some of the strains were more than 15 years old. Additional confirmation will be found in the tables.

Consideration of the data in the tables may be more revealing if the fat-splitting ability of each of the diverse groups which comprise the genus *Phytomonas* Bergey *et al.* is discussed in turn.

Xanthomonas. The yellow-pigmented, monotrichous or nonmotile, slime-forming, gram-negative, nonsporing, rod-shaped bacteria, which may cause disease in plants, were placed by Dowson (10) in the new genus, *Xanthomonas*. This genus, with certain exceptions, is synonymous with the subgroup, "Phytomonas proper," of the genus *Phytomonas* Bergey *et al.* (2), and with those species frequently referred to as the *Phytomonas campestris* group. For reasons advanced and reviewed by Dowson (10) and supported by the junior writer (3, 4), it seems desirable to refer to these organisms as *Xanthomonas* species, especially since Elliott (11) has pointed out that *Phytomonas* is a homonym.

The *Xanthomonas* species are actively lipolytic by the spirit-blue cottonseed-oil agar method. The data included in table 1, show that isolates of 21 of the 24 authentic species and varieties of *Xanthomonas* examined were able to decompose cottonseed oil. It should be noted that the previously mentioned observations of Jodidi (14) and of Castell (5, 6) concerning the lipolytic activity, respectively, of *X. pruni* and *X. campestris*, are thus confirmed. It is significant that *X. rubrilineans,* one of the few species in this

TABLE 1.—*The lipolytic activity of some strains of Xanthomonas on spirit-blue cottonseed-oil agar*

Species	Source	Lipolytic ability
Xanthomonas barbareae	WHB[a] BI, BR	Active
Xanthomonas begoniae	Oregon WHB 96	Active
" "	WHB	Active
Xanthomonas campestris	WHB 85, 86, R4	Active
" "	Oregon WHB C 22	Active
Xanthomonas campestris v. *armoraciae*	WHB HI	Active
Xanthomonas corylina	Oregon 5401, 5402, 5403	Active
" "	WHB 78	Active
Xanthomonas geranii	WHB G1, G2, G3, 87, 89	Active
Xanthomonas gummisudans	WHB 94	Active
Xanthomonas hederae	WHB 16	None
Xanthomonas holcicola	Elliott 102	Active
Xanthomonas juglandis	WHB 77	Active
" "	WHB 79	Slight
" "	Oregon 5080, 5171, 5184	Active
Xanthomonas malvacearum	Braun B87	None
" "	MPS[a] 1, 2, 3, 4	None
Xanthomonas papavericola	WHB 47, 48	Active
Xanthomonas pelargonii	WHB 100, WHB 92	Active
" "	Oregon WHB 93	Active
Xanthomonas phaseoli	WHB E2	Active
" "	Oregon WHB 5	Active
" "	Braun B16	Active
" "	Hedges	Active
" "	MPS 1941	Active
Xanthomonas phaseoli v. *fuscans*	WHB 8	Active
Xanthomonas phaseoli v. *sojense*	WHB 11	Active
Xanthomonas pruni	Oregon WHB 24	Active
" "	Braun B65, B67	Active
" "	Thornberry 1, 2, 3	Active
Xanthomonas rubrilineans	Braun B90	None
Xanthomonas translucens	WHB 61, 90	Active
Xanthomonas translucens v. *undulosa*	WHB 83, 91	Active
Xanthomonas vasculorum	WHB 49	Active
Xanthomonas vesicatoria	Pepper WHB 1, 2, 3, 4, 5, 6, 7, 99	Active
" "	Tomato WHB 13, 16, 38, VI	Active
" "	Unknown Ark	Active
Xanthomonas vesicatoria v. *raphani*	Lincoln	Active
Xanthomonas sp. (Kendrick's stock, *Matthiola incana,* pathogen)	WHB St1	Questionable
" "	WHB St2, St3	Active

[a] The designation WHB indicates the culture collection of the junior writer; MPS, that of the senior writer.

group that is not lipolytic, does not conform closely to certain other cultural characteristics of this genus.

Phytopathogenic Pseudomonas. The close relationship of the green-fluorescent, pigment-producing phytopathogenic bacteria now in the genus *Phytomonas* Bergey *et al.* to the saprophytes and animal pathogens now in Bergey's *Manual* in the genus *Pseudomonas* Migula has been shown by several workers. Rahn (15) has already pointed out the "practical impossi-

TABLE 2.—*The lipolytic activity of some phytopathogenic Pseudomonas on spirit-blue cottonseed-oil agar*

Species	Source	Lipolytic ability
Pseudomonas aceris	Ark	None
Pseudomonas alliicola	WHB[a] On1, AN, 1, 2, 4, R1, R2, R4	Active
Pseudomonas andropogoni	Braun B82	None
Pseudomonas angulata	Braun B99	None
Pseudomonas berberidis	WHB G35	None
Pseudomonas caryophylli	WHB RC, 3, 4, B, R2, R3, R8, RR7, RB, Car 1	Active
" "	WHB C, R4, Jones	Slight
Pseudomonas cichorii (endiviae)[b]	WHB G18	None
Pseudomonas coronafaciens	Elliott 75	None
Pseudomonas delphinii	WHB G36	None
Pseudomonas glycinea	WHB G8	None
Pseudomonas lachrymans	WHB G12	None
Pseudomonas lapsa	Ark	None
Pseudomonas marginata	WHB G40	Active
Pseudomonas maculicola	WHB G37, CI	None
Pseudomonas medicaginis	WHB G10	None
Pseudomonas medicaginis v. *phaseolicola*	WHB 1, G3, G29, G31, A, MPS 1941	None
Pseudomonas mors-prunorum	Braun B69	None
Pseudomonas pisi	WHB G32	None
Pseudomonas polycolor	WHB G11	Active
Pseudomonas primulae	Ark	None
Pseudomonas striafaciens	Elliott 70	None
Pseudomonas syringae	WHB G7, SyF, Syl	None
Pseudomonas syringae (vignae)[b]	WHB G15	None
Pseudomonas syringae (vignae v. *leguminophila)*	Braun B45	None
Pseudomonas syringae (cerasi v. *prunicola)*	Braun B38	None
Pseudomonas syringae (prunicola)	Braun B70	None
Pseudomonas syringae (holci)	WHB G28	None
Pseudomonas syringae (trifoliorum)	WHB G20	None
Pseudomonas syringae (cerasi)	WHB G21	None
Pseudomonas syringae (utiformica)	WHB G24	None
Pseudomonas tabaci	Penn. State	None
Pseudomonas viburni	Thornberry 1, 2, 3	None
Pseudomonas viridiflava	WHB, G4, G5	None
Pseudomonas viridilivida	WHB G23	None
Pseudomonas woodsii	WHB G34	None

[a] See footnote, table 1.

[b] Species names in parentheses are those synonyms under which the cultures were received.

bilities" involved in a separation of these two groups of microorganisms based solely on the character of plant pathogenicity. The comparative studies of Clara (7), Seleen (16) and others have demonstrated the cultural similarity of these groups, and a merger of the two genera has been recommended (1, 3, 10). In this study, the green-fluorescent, and certain related but nonpigmented, plant pathogenic bacteria will be referred to as species of *Pseudomonas.*

The strong lipolytic activity of a number of *saprophytic* species of *Pseudomonas* has been reported (5, 6, 8). The writers, also, have observed that 15 isolates of the saprophyte, *Ps. fluorescens,* were strongly lipolytic by the spirit-blue cottonseed-oil-agar technique. Accordingly, it may be surprising to learn from the data in table 2 that the majority of *phytopathogenic Pseudomonas* species studied did not split cottonseed oil by this method. Of the 27 species tested, isolates only of 4 species were lipolytic. Of these lipolytic plant pathogenic species, at least one, *Ps. polycolor,* is culturally so closely related to the lipolytic saprophyte, *Ps. fluorescens,* that any attempt at a separation, except on the basis of plant pathogenicity, is at present impossible. The other 3 lipolytic species, *Ps. alliicola, Ps. caryophylli,* and *Ps. marginata,* have never been known to produce a green-fluorescent, diffusable pigment, and, possibly, are not now classified correctly.

The Gall-forming, Rhizobium-like Group. The junior writer (3) pointed out the close relationship of certain of the gall-forming phytopathogenic bacteria to the Rhizobiaceae Conn and, later, Conn, Wolfe, and Ford (9) and Hofer (12) discussed the taxonomy of these species. The data in table 3 indicate that the twelve strains of tumefying *Phytomonas,* cultured on spirit-blue cottonseed-oil agar, showed no typical lipolytic reaction,

TABLE 3.—*The lipolytic activity of the gall-forming phytopathogenic bacteria and of some related Rhizobiaceae on spirit-blue cottonseed-oil agar*

Species	Source	Lipolytic ability
Phytomonas gypsophilae	N. Brown	None
Phytomonas pseudotsugae	Hansen	None
Phytomonas rhizogenes	Hildebrand	None
Phytomonas savastanoi	Braun B40	None
" "	C. O. Smith	None
Phytomonas savastanoi v. *fraxini*	N. Brown	None
Phytomonas tonelliana	C. O. Smith	None
Phytomonas tumefaciens	A.T.C. 6408	None
" "	Hildebrand B2, BP	None
" "	Braun B2, B6	None
Rhizobium leguminosarum (*Medicago*)	MPS[a]	None
Rhizobium leguminosarum (*Arachis*)	MPS	None
Chromobacterium violaceum	MPS	None
Chromobacterium ianthinum	MPS	None
Alcaligenes fecalis	MPS	None
Alcaligenes radiobacter	Hofer	None

[a] See footnote, table 1.

although certain cultures caused a transient bluing of the medium, the significance of which is not yet understood. The fact that 6 strains belonging to the 3 genera of the Rhizobiaceae were also without demonstrable fat-splitting ability may be a further indication of the postulated relationship.

The Corynebacterium (Gram-positive) and Phytomonas stewartii (Gram-negative) Groups. On the basis of his extensive studies, Jensen (13) considered that at least 4 species of plant-pathogenic bacteria belong in the genus *Corynebacterium* Lehmann and Neumann. It is likely that other gram-positive species included in Bergey's *Manual* (2) as part of "Appendix II" of the present genus *Phytomonas* are also species of *Corynebacterium;* these are so indicated in table 4. Since the taxonomic position of the gram-negative species, *Phytomonas stewartii, P. tardicrescens,* and *P. manihotis,* is rather uncertain, it will not be discussed further here, and the name *Phytomonas* is retained.

TABLE 4.—*The lipolytic activity of the phytopathogenic corynebacteria (gram-positive) and of the Phytomonas stewartii (gram-negative) group on spirit-blue cottonseed-oil agar*

Species	Source	Lipolytic ability
Corynebacterium fascians	Oregon P1, P2	Slight or questionable
" "	Hildebrand R, S	Slight
Corynebacterium flaccumfaciens	WHB[a] S1, S2, S3, S4, S5, S6, S7, S9, S20, S31	None
Corynebacterium insidiosum	Oregon WHB S24	None
" "	Fitzpatrick A11, A21, A34, A39, A41, A42, A50, A54, A55, A57, A74, A77, A80, A81, A89, A91, A97, A99	None
Corynebacterium michiganensis	WHB S11, S18, S29	None
Phytomonas stewartii	Braun B93, B95	None
" "	Elliott 403517, 403558	None
" "	Lindstrom—virulent 3152, 3153, 3155, 3156	None
" "	Lindstrom—avirulent 3151, 3154	None
" "	WHB S13, S15, S16	None
Phytomonas manihotis	WHB 1, 2, 4, R1, R2, R3, R4	Slight or questionable
Phytomonas tardicrescens	WHB S26	None

[a] See footnote, table 1.

As shown in table 4, none of the phytopathogenic corynebacteria or of the gram-negative *Phytomonas stewartii* group are actively lipolytic by the spirit-blue-agar method. However, it should be noted that *Corynebacterium fascians* and *Phytomonas manihotis* show a weak or questionable reaction.

SUMMARY

The spirit-blue-agar technique was used for studying decomposition of cottonseed oil by phytopathogenic bacteria. Two hundred and six cultures belonging to 65 species and varieties of the genus *Phytomonas* Bergey *et al.* were examined by this method. The reaction on spirit-blue cottonseed-oil agar may be of value in classifying the plant-pathogenic bacteria, since isolates of any one species give similar reactions regardless of source or age of the culture. Moreover, each of the groups comprising the genus *Phytomonas* Bergey *et al.* appears to have a tolerably characteristic ability to decompose fat as shown by this technique. Of 24 *Xanthomonas* species and varieties tested, 21 are actively lipolytic. Isolates of only 4 of the 27 species of phytopathogenic *Pseudomonas* are lipolytic; further study may reveal other, more correct, relationships for these lipolytic species. None of the 18 isolates of gall-forming plant pathogens and related Rhizobiaceae thus examined gave a typical lipolytic reaction. None of the tested species of phytopathogenic *Corynebacterium* or of the *Phytomonas stewartii* group are actively lipolytic, although in two species there was recorded a slow or questionable reaction. The taxonomy and nomenclature of the plant-pathogenic bacteria are discussed particularly in the light of this study.

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THE BROAD RING-SPOT VIRUS

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Because of the number of viruses occurring naturally on tobacco (*Nicotiana tabacum* L.), and the common use of this host as a test plant for many others, it may be of some value to describe more fully a new virus reported from this laboratory in 1938 (2). This virus, to which the common name broad ring-spot was applied, is also of some interest because of its natural occurrence in only a single instance on tobacco, a circumstance that may eventually have some bearing on theories concerning the origin of viruses. Not only did the virus fail to appear in any other fields surveyed in 1938, but it has not been found since 1938 in the same or other fields. In 25 years of fairly extensive surveys for virus diseases in Wisconsin tobacco fields, it is not recalled that similar symptoms have ever been noted, nor have they been seen in numerous tobacco fields of other States inspected by the senior writer. The virus, therefore, should be regarded as extremely rare on tobacco. Nothing is known of its natural occurrence on other host plants, many of which are now known to be susceptible to the virus by artificial inoculation.

Broad ring spot was found in a 6-acre field of tobacco near Edgerton, Wisconsin, on a crop nearing the topping stage. About 30 per cent of the plants in the field showed distinct symptoms. The disease was at first thought to be ordinary tobacco ring spot (5), known not to occur naturally in Wisconsin. Specimens of the leaves were taken to the laboratory and photographed (Fig. 1, *A*). Young tobacco and tomato plants, in the greenhouse, were inoculated with extracts. Good infection and symptoms were readily obtained on both hosts (Fig. 1, *B* and *C*). Since ordinary tobacco ring-spot virus is not known to infect tomato by the extract method of inoculation, more detailed comparative studies were undertaken, particularly with Prices' tobacco ring-spot No. 2 (3), Imle and Samson's tomato ring-spot (1), and Zaumeyer's alfalfa-mosaic virus (6). These viruses were secured either directly or indirectly from the above-named investigators.

SYMPTOMS

The symptoms of the broad ring-spot disease are not sufficiently distinctive to permit satisfactory or reliable identification of the causative virus on this basis alone. The relatively wide band of chlorotic or necrotic tissue comprising the circumference of the ring is, compared to other ring spots, often distinct, but the variability is too great to allow one to attribute much diagnostic significance to this character.

On young tobacco plants (variety Havana No. 38), the first symptoms on inoculated leaves appear as indistinct yellowish spots in 4 to 6 days.

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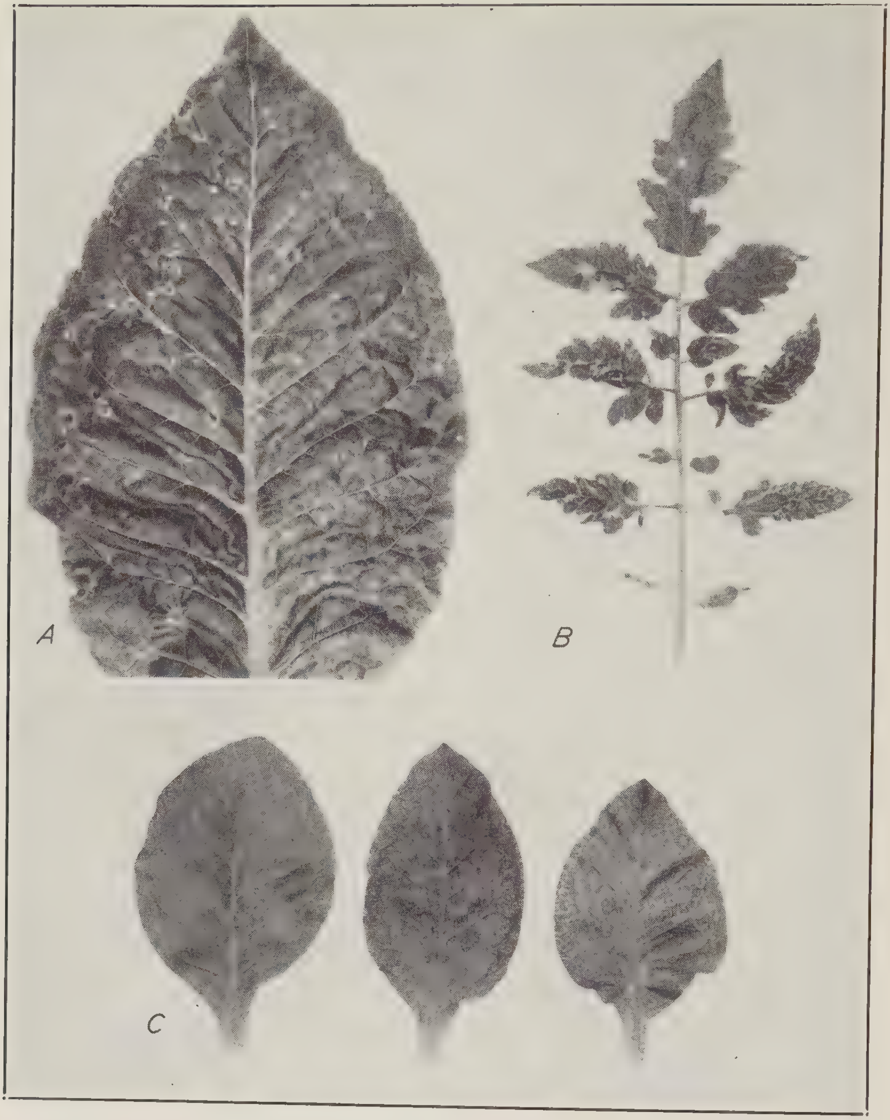

FIG. 1. *A*. A tobacco leaf from the original collection of broad ring spot from the field. These symptoms were characteristic on about 30 per cent of the plants in a 6-acre field. *B*. Artificial inoculations of broad ring spot to tomato under greenhouse conditions yield characteristic but less distinct ring-spot symptoms and considerable distortion. *C*. Artificial inoculations with broad ring-spot virus by the wiping method produces few but good inoculative symptoms on tobacco (leaf at left) but more abundant and distinct systemic symptoms (leaves at center and right).

These local areas may show a few fine, concentric, necrotic lines (Fig. 1, *C*), but more often the lesions become chlorotic rings. Systemic symptoms develop in about 7 days. The young, systemically infected leaves may at first become slightly puckered along the veins, resulting in some malformation (Fig. 2, *A*). Small chlorotic rings then appear, often 2 or more being ar-

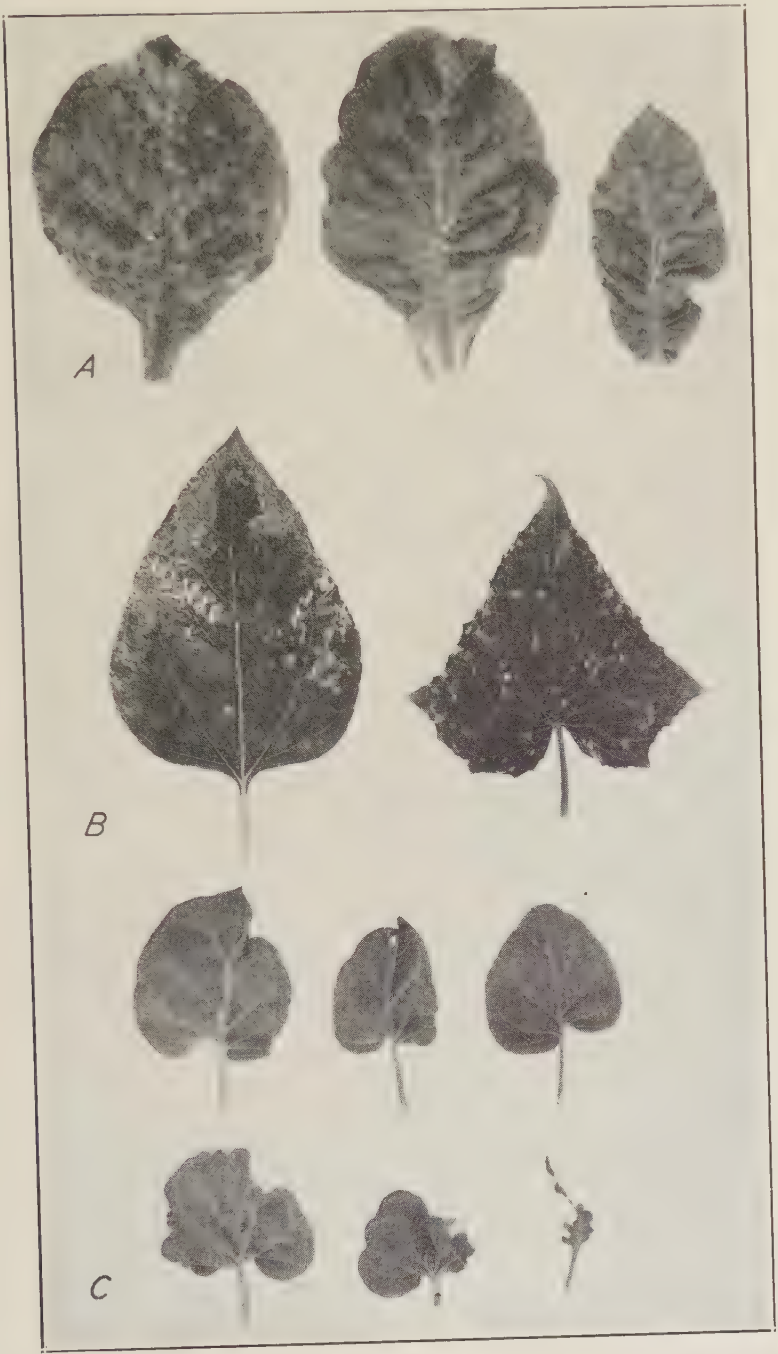

FIG. 2. *A*. Broad ring-spot virus often produces some puckering and malformation on systemically infected tobacco leaves. *B*. Systemic symptoms of broad ring spot on artificially inoculated sunflower (left) and cucumber (right) leaves. *C*. The symptoms of broad ring spot on *Nicotiana glutinosa* are not characteristic of those on other hosts, but the leaves are often variously deformed as shown here.

ranged concentrically (Fig. 1, *A*). Their location seems to bear no definite relation to the veins, as contrasted to ordinary tobacco ring-spot symptoms. Systemic symptoms appear on 2 or 3 young leaves. Succeeding new leaves, as they are produced, are apparently healthy. This recovery, however, is not permanent. Following the development of 3 or 4 apparently healthy leaves, it is not unusual for a young leaf to again develop symptoms especially on the tip half of the leaf. It has been shown also that the virus is present in the leaves showing no symptoms. Such leaves do not yield symptoms at the point of inoculation with broad ring-spot virus, although they may be infected with the viruses of potato ring spot, ordinary tobacco ring spot, tobacco ring spot No. 2, and alfalfa mosaic.

In the early fall and late spring, when greenhouse temperatures are higher, the chlorotic type of ring spot is partly replaced by fine, brown, necrotic rings. This type of symptom was characteristic also of the appearance of the disease in the field (Fig. 1, *A*). On tomato (variety John Baer), the virus also induces definite chlorotic and necrotic ring-spot symptoms. The rings, however, are somewhat broader than on tobacco leaves, and follow the veins to a greater extent (Fig. 1, *B*). Tomato leaflets usually are distorted by the virus to a much greater extent than are tobacco leaves.

TRANSMISSION OF THE VIRUS

The broad ring-spot virus is easily transmitted by mechanical inoculation. In some early trials, higher percentages of infection were obtained with diluted extracts of the virus if the leaves to be inoculated were first lightly dusted with carborundum powder. Consequently, carborundum powder was used in all property studies.

No extensive trials have been made in an effort to find insect vectors of the broad ring-spot virus. In 7 trials, however, involving the transfer of several thousand aphids (*Myzus persicae* Sulz.) from diseased plants to a total of 35 healthy tobacco plants no transmission of the virus by this insect has occurred.

PROPERTIES OF THE VIRUS

Property trials were made on the fresh extract of diseased tobacco plants. In table 1 is shown a summary of the data on the thermal death-point, longevity *in vitro,* and tolerance to dilution.

Thermal death-point determinations were made by heating 1 cc. of fresh leaf extract in a thin-walled test tube immersed in a water bath for 10 minutes. The virus never survived 54° C. for 10 minutes, but occasionally remained infective after being held at 52° C. for 10 minutes. The thermal death point is therefore about 54° rather than 52° C. as stated in the earlier abstract (2). The virus survived for 42 hours, but not for 48 hours, *in vitro* at room temperature. In other trials, extracts held at 4° C. or frozen at about $-2°$ C. retained their infectivity for 16 days. Extracts diluted to 1:1000 with tap water occasionally caused infection, while greater dilutions

TABLE 1

Summarized results from tests on certain physical properties of the tobacco broad ring-spot virus on tobacco

Thermal death-point

Temp. °C. of exposure of virus for 10 min.	None	48°	50°	52°	54°
No. plants inoculated	55	45	55	55	45
No. plants infected	55	22	12	6	0

Longevity *in vitro*

No. of hours of aging of virus at room temperature	None	30	36	42	48
No. plants inoculated	70	35	70	55	30
No. plants infected	70	20	15	10	0

Tolerance to dilution

Amount of dilution of virus extract with water	None	1: 250	1: 500	1: 750	1: 1000	1: 2500
No. plants inoculated	50	20	40	40	45	25
No. plants infected	50	15	16	14	6	0

did not. The dilution end point is therefore beyond 1: 1000 instead of about 1: 750, as stated in the preliminary abstract (2).

Filtration trials were made on extracts partly clarified by low-speed centrifugation, followed by dilution with an equal volume of distilled water or buffer solution. In 4 trials, with the extract at the normal pH of 6.3, adjusted to pH 4.8 and again to pH 8.0, the virus failed to pass a Berkefeld V filter. The use of Berkefeld N, Chamberland F filters, and Seitz E. K. filter pads in other tests also gave negative results. The unfiltered control extracts gave 100 per cent infection in all instances. The broad ring-spot virus, therefore, is evidently not readily, if at all, filterable.

HOST RANGE OF THE VIRUS

Actively growing young plants of many plant species have been inoculated with the broad ring-spot virus. Seven to 14 days after inoculation, whether or not symptoms were secured, subinoculations were made back to healthy tobacco plants. The virus was reisolated from the following species distributed through the families shown:

Amaranthaceae: *Amaranthus retroflexus* L.; Boraginaceae: *Cynoglossum amabile* Stapf. and Drum.; Chenopodiaceae: *Chenopodium album* L., *Spinacia oleracea* L.; Compositae: *Calendula officinalis* L., *Callistephus chinensis* Nees., *Helianthus annuus* L., *Zinnia elegans* Jacq.; Cruciferae: *Brassica arvensis* Kuntze, *B. nigra* Koch, *B. oleracea* L., *Bursa bursa-pastoris* (L.) Britton; Cucurbitaceae: *Cucumis sativus* L., *Cucurbita maxima* Duchesne; Euphorbiaceae: *Acalypha virginica* L.; Leguminosae: *Dolichos lablab* L., *Phaseolus vulgaris* L., *Pisum sativum* L., *Trifolium incarnatum* L., *Vigna sinensis* Endl.; MALVACEAE: *Hibiscus esculentus* L.; POLYGONACEAE: *Fagopyrum esculentum* Gaertn., *Rumex crispus* L.; PORTULACACEAE: *Portulaca oleracea* L.; RANUNCULACEAE: *Delphinium* sp.; SCROPHULARIACEAE: *Antirrhinum majus* L.; SOLANACEAE: *Datura stramonium* L., *Lycopersicum esculentum* Mill., *Nicandra physalodes* (L.) Pers., *Nicotiana angustifolia* Ruiz. and Pav., *N. glutinosa* L., *N. rustica* L., *N. sylvestris* Speg. and Comes., *N. tabacum* L., *Petunia violacea* Lindl., *Physalis* sp., *Solanum melongena* L., *S. nigrum* L., *S. tuberosum* L.; TROPAEOLACEAE: *Tropaeolum majus* L.; UMBELLIFERAE: *Apium graveolens* L.

Symptoms of broad ring spot varied from pronounced chlorotic rings, as on sunflower (Fig. 2, *B*, left), to scattered yellow spots, as on cucumber (Fig. 2, *B*, right). The more usual type of symptom on the majority of species was, however, a mild vermiculate pattern of chlorosis. *Nicotiana glutinosa* (Fig. 2, *C*) and *N. sylvestris* leaves were much distorted. Infections on squash and potato remained localized, but the virus became systemic on all other hosts tested under greenhouse conditions. Some of the hosts, although yielding definite symptoms of broad ring spot, permitted only with much difficulty transfer of the virus back to tobacco. Such sources of poor inoculum are represented by *Cucumus sativus, Datura stramonium, Solanum melongena, Chenopodium album,* and *Spinacia oleracea.*

No symptoms developed and no virus could be recovered from inoculated plants of *Beta vulgaris* L., *Citrullus vulgaris* Schrad., *Medicago sativa* L., and *Melilotus alba* Desr.

DISCUSSION

On the basis of symptoms only, broad ring spot of tobacco might easily be confused with other virus diseases of the ring-spot type. The wide host range of this virus also bears some resemblance to those of ordinary tobacco ring-spot (5), tobacco ring-spot No. 2 (4), and the alfalfa-mosaic (6) viruses. There are however some significant differences, as, for example, on tomato and spinach, which are both susceptible to the broad ring-spot virus by mechanical inoculation, but the former host is not susceptible to either ordinary tobacco ring-spot or alfalfa-mosaic virus, and the latter host is not susceptible to tobacco ring-spot No. 2 virus by the same method of inoculation. Significant differences also exist in the physical properties of the above-named viruses, particularly in the thermal death point, which is somewhat lower for the broad ring-spot virus than for either of the tobacco ring-spots or the alfalfa-mosaic viruses. These factors, together with the proven failure of these viruses to yield any cross immunity in the plants, may be regarded as sufficient evidence that broad ring-spot virus is a new one rather than a virus strain, although it is perhaps much more closely related to the viruses with which it has been compared in this investigation than to any other known viruses.

The rather unusual circumstance of the virus being found only once under natural conditions and then in comparative abundance, as described in the introduction of this paper, suggests many possible interesting interpretations.

There is little probability in our opinion that the virus arose in Wisconsin by variation from ordinary tobacco ring-spot or other viruses of tobacco. The most logical explanation is that the virus existed previously in some unknown host plant in the vicinity of the seed beds or the field and was transmitted to the tobacco plants either mechanically or by an unknown and perhaps rare insect vector. It also may be assumed that the combination of circumstances that brought about the 1938 epidemic was due to a rare proximity of the hosts involved, or that the new virus cannot or has not yet become established on tobacco as a new host because of still other environmental conditions.

The discovery of new or undescribed viruses in nature, in field crops, or under greenhouse experimental conditions is now somewhat frequent, largely because more attention is given to the subject. It is quite likely that a serious attempt to locate and determine new viruses in nature, disregarding their frequency or economic importance, would yield a considerable number of new and distinct forms. The finding of new strains of known viruses is less significant because the origin of such variants is well established.

The problem of the origin of viruses may be perhaps more advantageously investigated with such rare viruses as broad ring-spot than with such a commonly prevalent form as the ordinary tobacco-mosaic virus. Either the numerous viruses already known (and perhaps a greater number yet to be determined) have come down through the ages, or are being developed more currently, descent and evolution being independent of any genetic mechanism yet defined.

SUMMARY

A description is presented of a new virus found on tobacco in the field. It has been given the common name "broad ring-spot" to distinguish it from other viruses producing similar types of symptoms.

The virus is transmissible to several other plant families, its thermal death-point is 54° C., maximum longevity *in vitro* about 42 hours, and the dilution end point slightly above 1 in 1000. It is apparently not filterable by ordinary methods, and is not transmitted by the common peach aphid. The properties and other characteristics are sufficient to distinguish the broad ring-spot virus from certain other viruses with which it has been compared.

The broad ring-spot virus, although too rare to be of any practical significance, is of some special interest to those working on problems relating to the origin and to the distribution of viruses in nature.

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THE GENUS CEREBELLA CESATI—ITS BIOLOGIC STATUS AND USE

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The genus *Cerebella* was described by Cesati in 1851 from material collected on *Bothriochloa ischaemum* in Northern Italy, and was for many years regarded as a true smut, closely allied to *Thecaphora* or *Urocystis*. In 1902 Soccardo transferred the genus to the Deuteromycetes, but the true relationship between *Cerebella* and the host plant has not been recognized by a number of workers in various countries, who have regarded *Cerebella* as pathogenic and in itself responsible for damage to the spikelets of grasses. Others have recognized the association of *Cerebella* with the honey-dew (sphacelia) stage of ergot, on which it grows saprophytically, forming a dark-brown or black convoluted stroma between the glumes of the grass spikelets. Cesati apparently realized the association of *Cerebella* with the ergot of *Bothriochloa* (due to *Claviceps pusilla*, described by Cesati in 1861 after Tulasne's elucidation of the life history of *Claviceps* in 1853), for, in his description of the genus *Cerebella*, is the phrase ". . . *verisimiliter ex sclerotio sphaceligeno oritur*." Subrmaniam (21), in describing a number of new species of *Cerebella* from India, mentioned that the genus was commonly found associated with species of *Sphacelia* on Gramineae. But, from his remark that one species of *Cerebella* produced black swellings on the grain of *Sorghum vulgare*, and the fact that he described a separate species for each host plant, it seems that he had no clear idea of the true relationship between *Cerebella* and the plants on which it is found. Recently Subramaniam has reported that all *Cerebella*-infected plants examined in 1921 showed also the conidia of ergot.[1]

McDonald (14) reported that an experimental plot of *Sorghum* in Kenya in 1923 was attacked by *Cerebella sorghi-vulgaris*, which had not previously been reported from Africa. Later, he announced that inoculation experiments with *Cerebella* on native sorghum had given positive results, but only when the heads were exuding a sugary secretion. This had been observed only in cold weather, and McDonald was uncertain whether or not it was due entirely to climate (15). There is but little doubt that the "sugary secretion" observed by McDonald was the sphacelia stage of a species of *Claviceps*, a disease known on *Sorghum* in India (18) and Africa (23). Wilkinson (24) regarded *Cerebella* as "a potential disease of cultivated graminaceous crops" in Kenya in 1926. Ajrekar (1) concluded that "*Cerebella* grows as an epiphyte or parasite on the sphacelia stromata or sclerotia of *Claviceps*"; but Rhind (19) recognized *Cerebella* as a saprophyte of ergot honey-dew. Gonçalvez (10) has recently reported *Cerebella* associated with the sphacelia of *Claviceps* on several hosts in Brazil, and he considered that *Cerebella* was parasitizing the ergot and not the higher host plant.

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[1] Privately communicated.

From Kenya, Gold Coast, and Southern Rhodesia there are several records of diseases of grasses, such as false smut and ear mould, said to be due to *Cerebella,* with no suggestion of any association with ergot (16, 6, 12, 13). Recently, Hopkins[2] has stated that, although *Cerebella* on various grasses is listed among the diseases of Southern Rhodesia, it has always been recognized there as a saprophyte. But yeast, *Fusarium,* and *Cladosporium* are found as saprophytes of ergot honey-dew too, and there is little justification for listing *Cerebella* instead of *Sphacelia* sp. or *Claviceps* sp. in host indexes. Hansford (11), in a list of diseases in Uganda, records *Cerebella* as a disease of several species of grasses, and for some other grasses mentions an association of *Cerebella* with *Sphacelia* sp. In practically every case the host given for *Cerebella* or some closely related host species has been recorded as ergotized elsewhere.

In Queensland, *Cerebella* was first recorded in 1887 when *Cerebella paspali* Cke. and Mass. (*Cerebella inquinans* (Berk. and Br.) Sacc.) was described from *Paspalum orbiculare* Forst. (*P. scrobiculatum* L., of early Australian authors), the fungus then being regarded as a smut allied to *Urocystis* or *Thecaphora* (8). Later, *Cerebella* was recorded from *Themeda australis* and *Heteropogon contortus* (4). The only other Queensland records of *Cerebella* are to be found in the Herbarium of the Plant Pathology Section of the Queensland Department of Agriculture, where several specimens having an encrustation of *Cerebella* on the glumes have been labelled "Smut," and in one case, *Thecaphora* sp. All of these specimens have shown ergot conidia beneath the superficial *Cerebella* when teased up in water on a slide.

Since May, 1940, collections of *Cerebella* on 13 species of grass have been made in southeastern Queensland, and an examination of these has shown that the conidia of *Claviceps* are always present in abundance in the same spikelet as the *Cerebella.* The same host species also have been found ergotized and quite uncontaminated with *Cerebella. Cerebella* from each host has been isolated and grown in pure culture on potato-dextrose agar where the characteristic cerebriform stroma of the genus is produced within a few days. No morphological differences between the isolations from 13 different hosts have been detected, the species being determined as *Cerebella inquinans* (Berk. and Br.) Sacc. This is in marked contrast to the 8 species of *Cerebella* recorded from 15 host species by Subramaniam. In culture *Cerebella* grows well on potato slices and on potato agar containing sucrose, glucose, or honey, the fungus producing spores readily on each of these. Inoculation of *Paspalum dilatatum* and *P. orbiculare* with a suspension of spores of *Cerebella* taken from a pure culture gave negative results; but inoculation of *P. dilatatum* with a mixture of conidia of *Claviceps paspali* and *Cerebella inquinans* produced an exudation of honey-dew after seven days, and the typical stromata of *Cerebella* appeared on the ergotized spikelets 2–3 days later. When a suspension of *Cerebella* spores in diluted honey was used for inoculation, a very slight superficial growth of that fungus occurred on the glumes, but there was no sign of parasitism by *Cerebella* and no interference with seed production.

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[2] Privately communicated.

Taking the evidence from the literature in conjunction with the results quoted above, it can be said that *Cerebella* is no more than a saprophyte that finds any carbohydrate-rich substratum suitable for growth. In this connection it is interesting to note that, in Brazil, *Cerebella* sp. has been found accompanying the attacks of the coffee-berry borer (3). In Europe, *Cerebella italica* is known to occur on the seeds of the chestnut and the oak, and *C. negeri* on the seeds of *Abies* (20).

No evidence has been found of any parasitism of *Claviceps* spp. by *Cerebella*, as suggested by Ajrekar (1) and Gonçalvez (10). In the very few cases seen where *Cerebella* was present after sclerotium development began, the *Cerebella* was found to be quite independent of the sclerotium and flourished only round the edges of the glumes, where a certain amount of the sugary secretion remained. In the majority of *Cerebella*-infected plants, development of sclerotia had not taken place before the spikelets were overgrown by the saprophyte.

The need exists for a revision of the species of *Cerebella*, of which, very probably, too many have been described. Ellis and Everhart (9), in describing *Cerebella spartinae* (regarded by them as a smut) from the United States (U.S.A.), refer to the stromata on *Spartina* as "subconfluent, extending along one side of the spike," and they remark that *C. spartinae* differs from *C. andropogonis* in habit. Differences in shape and position of stromata must be expected in a fungus that grows on ergot honey-dew that may be smeared about on the inflorescence. Other workers have followed Subramaniam, and, where possible, have determined their species of *Cerebella* according to the host plant on which it is found, *e.g.*, *C. panici* on *Panicum*, *C. cynodontis* on *Cynodon*. The arbitrary nature of this practice usually is recognized, but until the genus is monographed and good specific characters established, precise specific names cannot be applied in every case. Probably a number of the 18 species of *Cerebella* that have been described will be found to be synonymous with the early species, such as *C. andropogonis* and *C. inquinans*.

DISCUSSION

Several important points arise from an exact knowledge of the relation existing between the higher host plant and *Cerebella*.

Firstly, *Cerebella* should be removed from the host indexes, *e.g.*, the host indexes of India (7), of Queensland (5), of Southern Rhodesia[3] (12), and of Uganda (11), and *Sphacelia* sp. substituted, followed by a serious attempt to discover which species of *Claviceps* is responsible. (This is now being done in Southern Rhodesia and Queensland.) It is evident that there is a wide host range of ergot in Africa, India, and southeastern Asia; and the possibility of introducing new species of ergot to Australia or other countries in imported seed must not be overlooked. The record of *Cerebella andropogonis* on *Brachiaria* sp. by the U. S. Department of Agriculture Plant

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[3] See footnote 2.

Quarantine and Control Administration (2) serves to emphasize the necessity for carefully examining imported seeds to prevent the introduction of ergot. Where *Cerebella* is seen, it is a warning against ergot, which may not be detected if the sclerotia are small and similar in shape to the seeds.

Secondly, *Cerebella* provides a natural control of ergot by inhibiting sclerotia formation, a fact already noted by Rhind (19) and Ajrekar (1). Early in the season *Cerebella* is not much in evidence, but, from January onwards, it has been observed in ever-increasing abundance on *Paspalum dilatatum;* it is also very common on *Bothriochloa* spp. and *Dichanthium* spp., which are attacked by *Claviceps pusilla* Ces. in Queensland. On an area of about half an acre of *P. dilatatum* at Kangaroo Point, Brisbane, almost every ergotized spikelet is now (April, 1941) infected by *Cerebella,* and it is practically impossible to find sclerotia on the area. A restriction of sclerotia formation to early summer, at least in some seasons, must be an important means of reducing the amount of inoculum available in the next spring, and may, to some extent, determine whether or not an epiphytotic of ergot will occur in the following season. Cessation of development of *Claviceps* following *Cerebella* infection is probably due to the effect of "staling" products produced during the growth of the *Cerebella* in the honey-dew.

Thirdly, *Cerebella* is a good field indicator of the presence of ergot. Recently a number of new ergot hosts have been found in Queensland, several of which might have been overlooked were it not for the conspicuous growth of *Cerebella* on the inflorescences. Bulk collection of inflorescences in the neighborhood of *Cerebella*-infected plants usually provides plenty of sclerotia and honey-dew of *Claviceps* for further study.

Fourthly, the records of *Cerebella* provide valuable evidence concerning the history of ergot in Queensland and other countries. *C. anthaenantiae, C. panici* and *C. sorghi* were described as smuts by Tracy and Earle in America in 1899 (22), and in view of the known occurrence of ergot on cultivated and native species of *Sorghum* and on native grasses in Africa, India, and Australia, a knowledge of the ergot of *Anthaenantia, Panicum* and *Sorghum* in America would be of considerable interest. Of the ergotized native grasses recently found in Queensland, there are records of *Cerebella* on *Themeda australis*, and *Heteropogon contortus* in 1890 (4), and on *Paspalum orbiculare* in 1887 (8). Specimens of *Cymbopogon refractus* and *Bothriochloa decipiens*, collected in 1911 and 1912, respectively, are in the Herbarium of the Plant Pathologist, Department of Agriculture, Queensland. Both of these are infected with *Cerebella*, but show also conidia of *Claviceps*, though the former was previously identified as *Thecaphora* sp. These records show that the ergot of these grasses is of long standing in Queensland.

SUMMARY

The literature on the genus *Cerebella* has been reviewed, and further evidence is presented to show that *Cerebella* is a saprophyte of carbohydrate-

rich materials, the commonest substratum for the fungus being the sugary honey-dew secretion associated with the sphacelia stage of *Claviceps* spp. The need for a revision of the species of *Cerebella* is pointed out.

Four points arise from a knowledge of the relation between *Cerebella* and the plants on which it is found:

1. *Cerebella* should be removed from the host indexes, and *Sphacelia* sp. substituted.
2. *Cerebella* provides a natural control of ergot by inhibiting the development of sclerotia.
3. *Cerebella* is a good field indicator of the presence of ergot.
4. The history of ergot in a country may be traced through old records of *Cerebella* on grasses.

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A QUANTITATIVE METHOD FOR ASSAY OF TOBACCO-MOSAIC VIRUS PROTEIN

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For the most part the various workers interested in the serological aspects of the virus problem have been content with qualitative observations, or at best quasi-quantitative observations, even though it has been shown by Heidelberger and his coworkers, in a long series of papers published in the *Journal of Experimental Medicine,* that the reaction between an antibody and its antigen satisfies the criteria for quantitative chemical analysis. Kleczkowski[1] has recently shown that, in a general way, the serological behavior of tobacco-mosaic virus, of aucuba-mosaic virus, and of bushy-stunt virus is in each case typical of the behavior Heidelberger has observed in his work with various other proteins. The data presented herewith were obtained in another connection where the quantitative aspects of serology were not the prime consideration, but they serve to show that the reaction between tobacco-mosaic virus protein and its antibody may be subject to quantitative treatment, and in that respect serve as additional evidence in support of some of the views that Heidelberger holds, and they confirm some of the observations reported by Kleczkowski. The main consideration, however, is that they give us a convenient, rapid and reliable method of quantitative assay in studies involving small quantities of this particular virus protein. The method unquestionably may be extended to several other plant viruses.

The serum used in these studies was obtained in the usual manner by the intervenous injection of small quantities of the isolated virus protein, suspended in 0.87 per cent sodium chloride solution, into a rabbit at intervals of 3 days for several weeks. The serum, obtained by bleeding the anesthetized animal, was stored in a frozen condition after the red cells had been centrifuged out.

The virus protein used in the precipitin reaction was an electrodialyzed preparation of the isolated protein which was suspended in water to which enough sodium chloride had been added to give an 0.87 per cent solution. The dissolution of the antigen in 0.87 per cent NaCl solution is essential in quantitative work involving the precipitin reaction. The reason for this precaution is that the antibody is in the globulin fraction of the serum, and this particular serum fraction is precipitated unless the salt concentration is maintained in the various reactions in which it may be involved.

In the precipitin reaction carried out in these studies, 1 cc. of serum was added to a 25-cc. volumetric flask, and then a definite volume of the virus suspension was added, and finally enough 0.87 per cent NaCl solution was added to bring the volume up to the mark. The flask contents were thoroughly mixed and the flask was placed in an incubator at 37° C. for 24 hours. The antibody content of the reaction mixture was thus held constant and in no case was the virus protein added in sufficient quantity to completely deplete the antibody supply. That is, the antibody supply was always in excess. Thymol was used as a preservative.

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[1] Kleczkowski, A. Quantitative studies on the serological reactions of some plant viruses and of a pea nodule bacterium (*Rhizobium leguminosarum*). Br. Jour. Exp. Path. 22: 44–58. 1941.

The first determinations were nephelometric. In nephelometry and in colorimetry the transmission of light by a solution or suspension depends on the thickness, d, of the layer traversed, and on the concentration, c, of the colored component of the solution, or of the suspension. The quantitative expression relating these factors is known as Beer's law, and is written

$$I_d = I\ e^{-kdc}$$

where I is the intensity of the incident light and I_d is the intensity of the transmitted light.

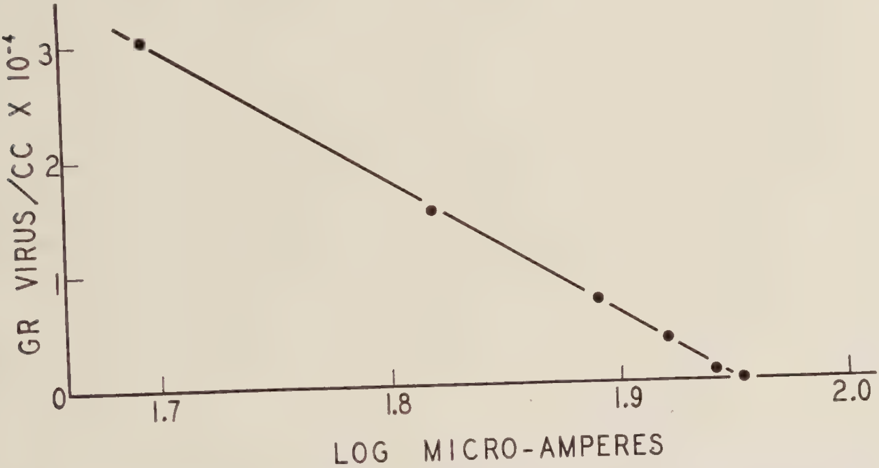

FIG. 1. The relationship between the amount of virus protein added to the serum in the reaction vessel and the nephelometric reading. Ordinate is the quantity of virus added to the serum; the abscissa is the current density observed in the nephelometer.

In nephelometry and colorimetry, as practiced, using the new photoelectric colorimeters, one varies c, keeping d constant, and observes the effect of that variation on I_d. If log I_d varies directly with c, where c is built up in the absorption cell of the colorimeter or nephelometer, one may conclude that Beer's law is followed. Beer's law has been found to be valid for many cases, but exceptions are known. Invariably, where exceptions to Beer's law are encountered in colorimetry, it has been found that the systems giving rise to the exceptions are systems where association or disassociation occurs, and where the degree of association or disassociation depends on the concentration. Difficulties in nephelometry are encountered when the precipitate formed in the absorption cell settles out too rapidly, or where the rate of growth of crystals or aggregates, once the nuclei have been formed, is great. Given the situation, however, where Beer's law is followed, one may conclude without much hesitancy that the reaction involved in the absorption chamber, giving rise to the component that is being measured, is stoichiometric. One may state in this connection that, although the virus protein particles do disassociate in dilute solution, the degree of dissociation in the dilutions studied does not seem to be great.

In the work reported in this paper, the conditions were such that the current output of the photronic cell in our colorimeter was proportional to the light intensity. In figure 1, the logarithm of the current density observed in the nephelometric determination is plotted against the quantity of virus protein added to the serum in the reaction vessel. The conclusion is obvious, and the nephelometric technique as outlined may serve as an accurate and rapid means of assay.

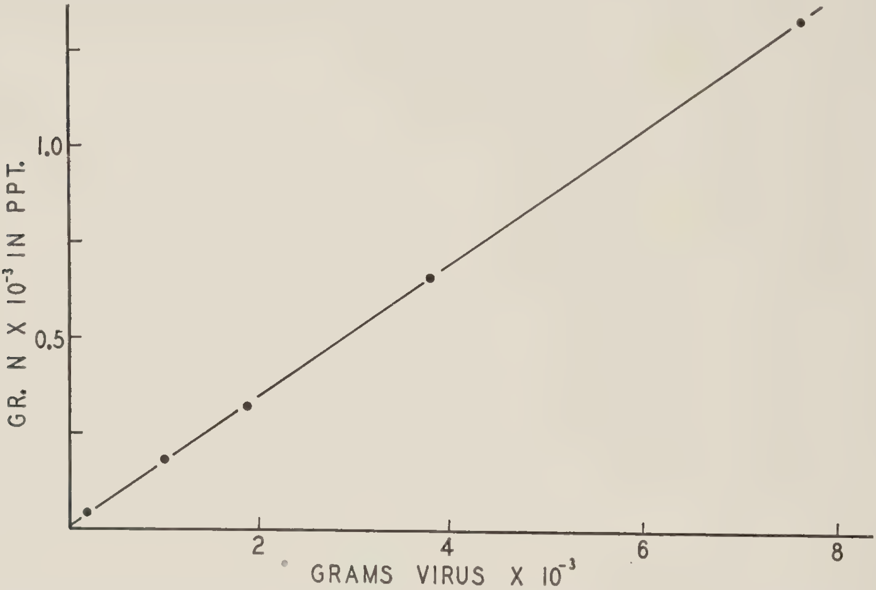

FIG. 2. The weight of nitrogen found in the precipitate formed in the precipitin reaction is plotted against the quantity of virus added to the serum in the reaction vessel.

Nitrogen and phosphorus determinations on the precipitate obtained from the precipitan reaction substantiate the view that, in the region of antibody excess, the reaction between the antigen and antibody is stoichiometric. The determinations were made in each case on the precipitate that had been packed in the bottom of a centrifuge tube and had been washed 3 times by suspension in 0.87 per cent NaCl solution, with subsequent sedimentation in the centrifuge. Nitrogen was determined by means of the Kjeldahl procedure. Data are presented in figure 2. The amount of nitrogen found in the precipitate is proportional to the amount of virus protein added to the reaction vessel.

Phosphorus determinations were made according to the Eddy and Deeds[2] method. Digestion was effected by means of a small quantity of H_2SO_4, together with a few drops of H_2O_2 (table 1). The quantities of phosphorus in these samples was very small, and consequently the error in phosphorus determinations was large, but the data indicate that the precipitation of the virus is substantially complete, and that there is no P in the antibody. One would conclude from these data that, in the region of antibody: antigen ratios studied, for each part of virus protein nitrogen in the precipitate there are 0.21 parts of antibody nitrogen. The ratio of 0.21 was obtained from the consideration that the ratio of phosphorus to nitrogen in the virus protein is 1:40, together with the analytical data for nitrogen and phosphorus in the precipitate. Kleczkowski reported a ratio of 0.25.

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[2] Eddy, C. W., and Floyd Deeds. A photoelectric method for the determination of phosphorus. Ind. and Eng. Chem. 9: 12–14. 1937.

TABLE 1.—*Phosphorus determinations from samples of virus precipitate as per the Eddy-Deeds method. Digestion was effected by adding H_2SO_4 and a few drops of H_2O_2*

G. virus protein used	G. P. found	G. P. expected[a]	Per cent recovery
7.6×10^{-3}	27×10^{-6}	30×10^{-6}	90
3.8	13	15	87
1.9	7.1	7.6	93

[a] G. of phosphorus expected on the basis of a determination of 0.4 per cent as the phosphorus content of the virus protein and on the assumption that precipitation of virus protein by the antibody is complete, and on the assumption that the antibody contained no phosphorus.

The following procedure is recommended in using serology in virus assay in the case of tobacco mosaic virus protein, where one is dealing with plant sap: Adjust pH of 50 ml. of virus-containing sap to some value between 4.5 and 3.5, and filter through a few grams diatomaceous earth (it has been observed that the adsorption on diatomaceous earth is complete at pH 4.5 or less). The filtrate is discarded. Suspend the filter cake in 25 ml. of water, cautiously adjust pH to 8.5, using NaOH, mix thoroughly and filter. Wash the filter cake at least 3 times with a few cc. of water. Combine the filtrate and washings in a 100-ml. volumetric flask, add enough NaCl to give 0.87 per cent solution, and finally add enough 0.87 per cent NaCl solution to bring the volume up to the mark, then mix. Add 1 ml. of serum containing the antivirus to 24 ml. of the virus extract, and after thoroughly mixing, store at 37° C. for 24 hours. In the event that the color originally present in the plant sap has not been entirely eliminated, centrifuge the virus-antivirus mixture strongly (3,000 × G) for about 30 minutes, decant, and suspend the precipitate in a known volume of 0.87 per cent NaCl solution. The last traces of color may be eliminated by a repetition of the sedimentation. The suspension is then placed in the nephelometer, and the reading obtained is referred to the calibration curve, where one may read off directly the amount of virus protein in the cell of the nephelometer.

The calibration curve is obtained by adding progressive amounts of the virus protein suspended in 0.87 per cent NaCl to successive volumetric flasks, each of which contains the same amount of serum, say 1 ml., and then bringing the volume up to the mark by means of 0.87 per cent NaCl solution. Mix the flask contents and store at 37° C. for 24 hours. Stir up the sediment before pouring the suspension into the nephelometer cell. The log of nephelometer reading is plotted against the amount of virus added to the volumetric flasks.

In the event that a suitable nephelometer is not available, or in the event color cannot be removed, virus protein assay that is more satisfactory than the local lesions method may be obtained from phosphorus determinations made on the washed precipitates, following the method of Eddy and Deeds and using an ordinary colorimeter. This suggestion comes from the consideration that the infectivity of the isolated virus preparations, as determined by the local lesions method, may vary from preparation to preparation by as much as 8000 per cent.

Several suggestions may be in order concerning quantitative serological work with the viruses in conjunction with the nephelometric technique. Where it is possible, the isolated virus protein should be used as the antigen. One reason for this suggestion is that complications may arise as a consequence of injecting unknown material into the experimental animal. By the way of an example, there is frequently enough solanin present in the sap of potato foliage to cause a fatal hemolysis if the whole sap is used for injection, and, at best, a non-fatal hemolysis results in a discolored and unsatisfactory product. A second reason for the suggestion that the isolated virus protein be used as the antigen is that during the manipulation of the protein involving the use of concentrated salt solutions and filtrations, the microorganisms originally present are largely eliminated, and the danger of septicemia is thereby reduced. A third reason for the suggestion is that one may then be in a position to take advantage of the specificity of the precipitin reaction and determine the virus protein content of a sample irrespective of its contamination with other proteins (neglecting cross reactions). In addition, the picture will not be complicated by the presence of a mixture of unknown antibodies.

It is recommended, finally, that a separate calibration curve be obtained for each serum sample.

SUMMARY

The precipitin reaction may be used in a rapid and accurate determination of tobacco-mosaic virus protein.

The amount of nitrogen and phosphorus found in the precipitate formed in the precipitin reaction is proportional to the amount of virus protein added.

The ratio of virus protein nitrogen to antibody nitrogen in the precipitate is 4.8.

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ITHACA, NEW YORK.

A METHOD OF GROWING PLANTS IN WATER VAPOR TO FACILITATE EXAMINATION OF ROOTS[1]

WALTER CARTER

(Accepted for publication October 11, 1941)

INTRODUCTION

In studies requiring the frequent and critical examination of roots, considerable mechanical injury and interference with growth result when the plants are grown in soil or even in sand and aerated water culture. The method of growing pineapple plants described herein was devised in order to facilitate the examination of the roots following infestation of the plants by mealy bugs.

THE WATER VAPOR METHOD

This consists of setting the plants in the perforated top of a simply constructed wooden box, in which is fixed an atomizing device similar to those devices used to keep vegetables fresh when they are displayed for sale, or in air-conditioning apparatus. These devices permit a very fine jet of water to impinge on a metal plate, which results in a steady drift of finely divided vapor. Domestic water pressure is adequate.

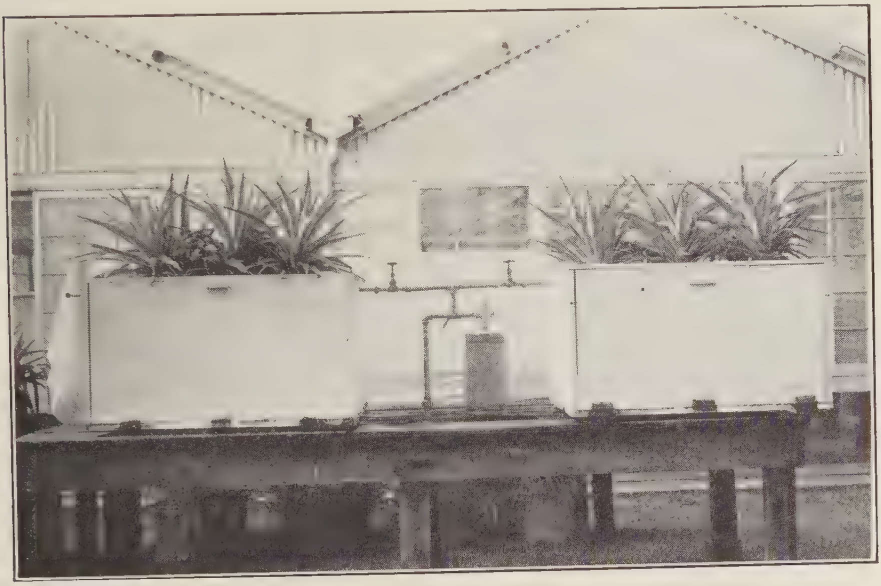

FIG. 1. Water-vapor culture box planted May 15, 1941, and photographed August 18, 1941.

To improve rooting and growth, a cylinder to contain concentrated nutrient solution is included in the system. For the 12 plants shown in figure 1, this cylinder, which contains 5 liters of the nutrient solution, is refilled every other day. The nutrient solution used is made up in the following proportions with the addition of an accessory solution of minor elements:

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[1] Published with the approval of the Acting Director as Miscellaneous Paper No. 37 of the Pineapple Research Institute of Hawaii, University of Hawaii.

	Cc. of ½ molar stock solution
KH_2PO_4	400
$Ca(NO_3)_2$	900
$MgSO_4$	450
$(NH_4)_2SO_4$	50
K_2SO_4	400

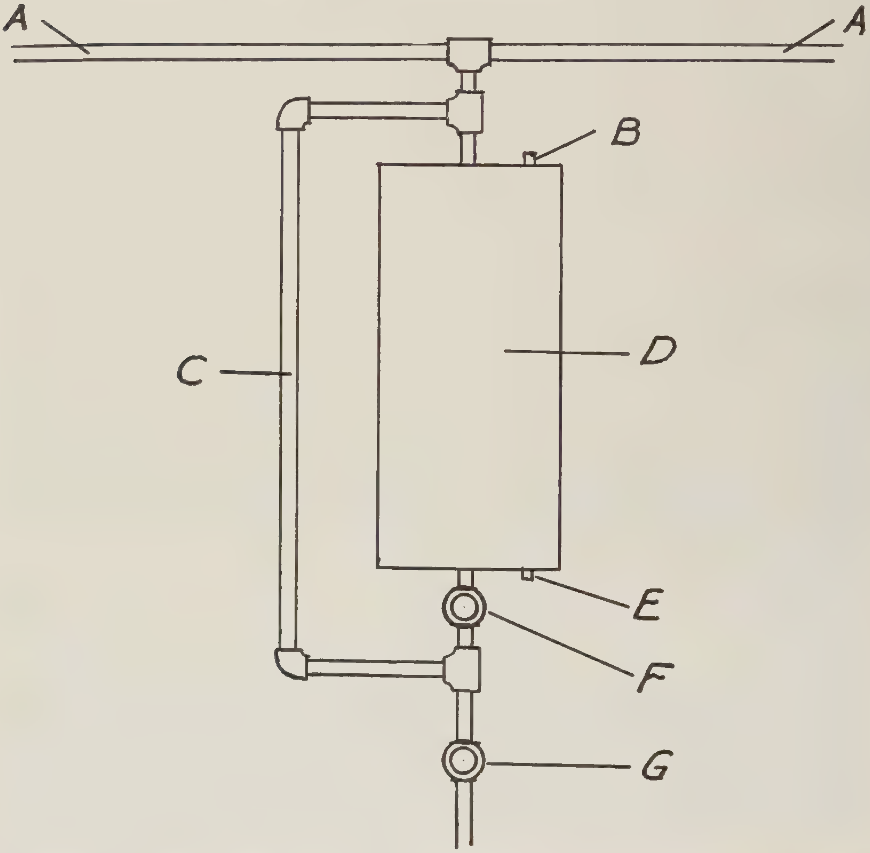

FIG. 2. Diagram showing device for introduction of nutrient solution into the water stream. A, to vapor boxes; B, filling plug; C, by-pass at full pressure; D, nutrient solution tank; E, drain plug; F, needle valve; and G, water supply valve.

This system does not provide a nutrient of constant concentration, but rather one that gradually dilutes. The discharge of nutrient from the cylinder was improved, following a suggestion by C. T. Schmidt,[2] when the water supply, instead of being led directly through the cylinder, is arranged as shown in figure 2. One arm of the T connection in the water line by-passes the water at full pressure around the cylinder; the other arm of the T connection leads to the cylinder, and the volume of water passing through the cylinder is reduced by means of a needle-point valve. In this way the exhaustion of the nutrient solution is usefully delayed.

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[2] Associate Entomologist, P.R.I.

Pineapple plants grown from vegetative slips by this method have grown at least as vigorously and as rapidly as in sand and water cultures in which the same nutrient solution was used. Root development is extensive (Fig. 3). The roots can be readily inspected and samples taken for study without damaging the balance of the root system.

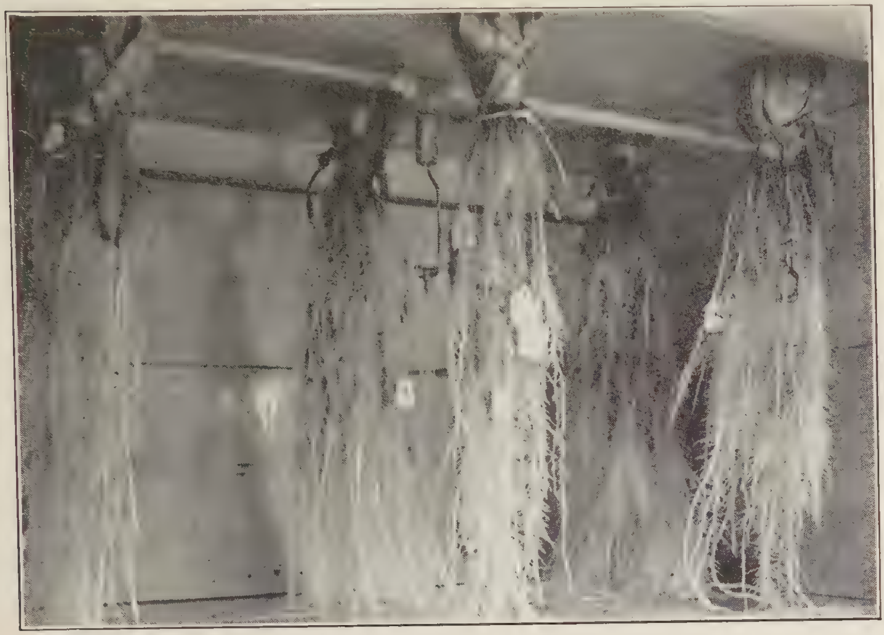

Fig. 3. Root development on 3-month-old plants in the water vapor culture box, August, 1941.

The vigor of growth obtained suggests the possibility of extending the use of this method, not only to the study of roots but also as a modification of the numerous methods employed in the water culture of commercial plants.

Pineapple Research Institute of Hawaii,
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CHLOROPICRIN AND ETHYLENE DICHLORIDE FOR ROOT-KNOT NEMATODE CONTROL

A. G. NEWHALL AND F. L. STARK, JR.

(Accepted for publication November 22, 1941)

In the short space of 10 years, chloropicrin has become the top-ranking chemical for treatment of soils infested with nematodes. We are indebted to a score or more of investigators for proving its efficacy and developing techniques for employing it. Particular attention might be called to the work of Johnson and Godfrey (5), Johnson (4), Neller and Allison (6), Godfrey (2), Newton, Bosher and Hastings (8), Howard, Stark and Smith (3), Young (10), Chitwood (1), and Taylor and McBeth (9), who have all tested it for root-knot control (*Heterodera marioni*).

Chitwood (1) recently pointed out that ethylene dichloride alone and in suitable mixtures with chloropicrin could be used at less expense and inconvenience to the operator than undiluted chloropicrin. Therefore, the authors compared chloropicrin alone at 2 cc. per injection with ethylene dichloride at 15 cc. and with a 1–9 mixture of the two at 10 cc. The work was done in a ground bed of a greenhouse built over fine sandy loam near Rochester, New York. The root knot had been progressively increasing in severity for several years, so that in 1940 only 2.75 lb. of tomatoes per plant were obtained from the spring crop, which is considered less than half of normal. The growers were about to try chloropicrin before installing steaming equipment, and readily agreed to turn over for experimentation an area of 1500 sq. ft. in one end of a 200-foot house, 30 ft. wide.

On September 26, 1940, 4 weeks after removal of the previous crop of tomato vines, 4 replicate plots of each of the 3 treatments plus 4 checks each $7\frac{3}{4} \times 12$ ft. in extent were laid out in a 4×4 Latin square (Table 1). The loose sandy soil was slightly moist and contained many partly decayed root galls of variable size up to $\frac{1}{2}$ in. in diameter. Soil temperature at a 3-in. depth was 67° F. Injections were made 4 to 5 in. deep with a "Larvajector"[1] at 10-in. intervals in rows 10 in. apart. After the injections were made and the holes tamped by stepping on them, water was liberally

TABLE 1.—*Plot arrangement and root scores indicating degree of nematode injury 11 months after treatment of soil with chloropicrin (Cp), ethylene dichloride (Ed), and a 1–9 mixture of these (Mix). N = no treatment*

N—59	Cp— 8	Mix— 2	Ed—33
Mix— 4	Ed—30	N—42	Cp—11
Cp— 9	Mix— 0	Ed—19	N—56
Ed—33	N—42	Cp— 5	Mix— 7

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[1] Hand-operated injector, holding approximately 2 quarts, made by Innis, Speiden & Co., Niagara Falls, N. Y.

sprinkled on all plots to seal the gas beneath the surface. In a similar manner the grower employed chloropicrin to soil in the remainder of the house and in another larger one.

Radishes were sown on the experimental plots November 23. These were harvested in February and were followed later by tomatoes. Yield records on the radish crop were kindly furnished the writers by the grower.[2] But, as might be expected from the fact that this is a low-temperature crop, no evidence of nematode activity was observed nor any significant differences found in total yields, due to treatments, as measured by the number of bunches harvested from each plot. The growers, however, noted that the radishes reached a stage large enough to harvest a few days sooner on the plots treated with the 1–9 mixture of chloropicrin and ethylene dichloride than on the others.

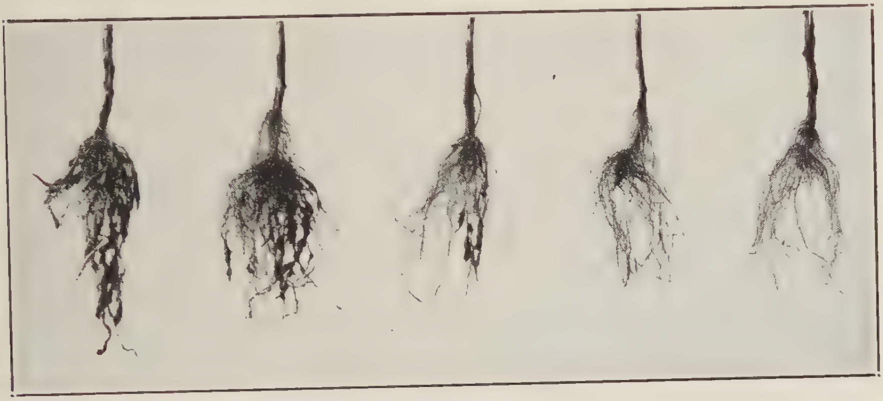

FIG. 1. Roots selected as standards by which to score those from the experimental plots. They represent (left to right) classes 4, 3, 2, 1, and 0.

On April 20, 1941, young tomato plants were set throughout the house 12 or 15 in. apart in rows approximately 2 ft. apart, so that 6 rows of 6 or 7 plants occupied each plot. Unfortunately, it was impracticable to obtain yield records of the tomatoes on the individual plots, but, by mid-summer, the check plots could easily be denoted by the stunted growth and yellowish color of these plants and conspicuous nematode galls on many of their surface roots.

All vines were loosened with a fork, pulled up, and carefully examined for nematode injury on August 23. The following scheme was employed to evaluate the different treatments for root-knot control. Individual plants, showing various degrees of nematode injury, were first selected for standards and placed in 5 classes ranging from no infection, which was assigned a zero, to very severe infection, which was assigned a value of 4 (Fig. 1). Fifteen roots were then taken from the center of each plot. Each was scored according to these standards; for example, if a root had been injured to approximately the same extent as standard number 2, it was scored as 2.

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[2] Acknowledgment is made to County Agent R. G. Palmer, for courtesies and help in carrying out this experiment.

Little difficulty was experienced in placing all 15 roots in the correct classes. The 15 scores for each plot were then totaled and this total considered as an estimation of the extent of nematode damage. The greater the amount of injury the larger the number. Applying these totals, it was possible to compare the relative values of the treatments for nematode control by employing the analysis of variance method for a Latin square. The results obtained are shown in tables 1 and 2.

It is evident that all treatments resulted in a very significant control of root-knot nematode. Moreover, chloropicrin alone and the mixture of chloropicrin with ethylene dichloride were superior to the ethylene dichloride alone. These differences all proved to be statistically significant with odds greater than 99:1. The slight difference in control between chloropicrin alone and the mixture did not prove to be statistically significant. The same conclusions would have been drawn on the basis of mere observation. (See Table 2 and Fig. 2.)

TABLE 2.—*Summary of root scores by treatments*

	No treatment	Ethylene dichloride, 15 cc. per injection	Chloropicrin, 2 cc. per injection	1–9 mixture of Cp. and Ed., 10 cc. per injection
Totals	198.0	115.0	33.0	13.0
Means	49.7	28.75	8.25	3.25

Least mean difference for significance, odds $\begin{cases} 99:1 = 20.045 \\ 19:1 = 12.070 \end{cases}$

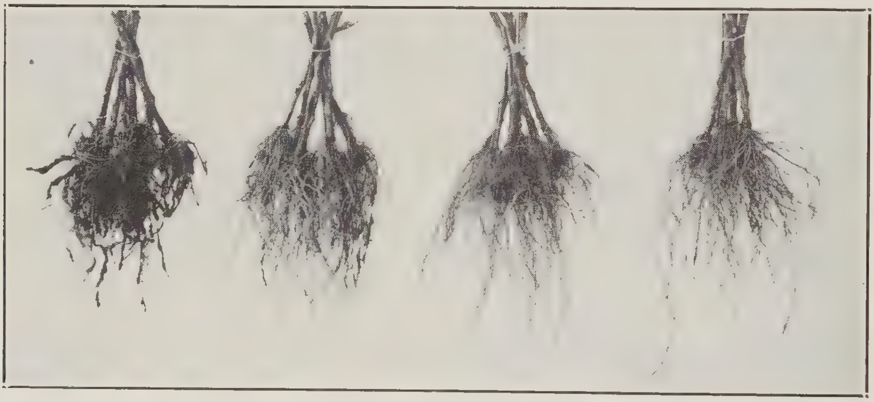

FIG. 2. Typical roots from plots receiving (left to right) no treatment, ethylene dichloride 15 cc., chloropicrin 2 cc., and the 1 to 9 mixture at 10 cc. per injection.

DISCUSSION

Some additional evidence of the value of the chloropicrin treatment was obtained from the growers' experience. As previously stated, they treated the remainder of the houses (14,000 sq. ft.) with chloropicrin in the same manner and at approximately the same rate as in the experiment. Before treatment their yields had dropped to a low of 2¾ lb. per plant in 1940 (less than half of normal for a spring crop). After soil treatment the 1941 crop yielded 7 lb. per plant. Although admittedly it is unscientific to compare the yield of one year with that of another, in this case there was no other factor that could presumably account for more than a small proportion of this 170 per cent increase in yield. Examination of the roots of over 1,000 plants revealed but very few nematode galls.

As judged by the amount of gall development on the experimental plots at the end of the season and by appearance of the plants during the last month, ethylene dichloride gave inferior control of nematodes to that obtained by either chloropicrin or the 1–9 mixture. However, without yield data we lack proof that ethylene dichloride is not economical, for Howard *et al.* (3) found that complete eradication of nematodes was not essential for normal yields of tomatoes. All that can be said now is that, until the present advance in price has receded, ethylene dichloride is definitely not recommendable. Final judgment should perhaps await further work on its fungicidal efficiency, which the writers believe is less than that of chloropicrin.

The cost of treatments may be summarized as follows: chloropicrin with 2-cc. injections 10″ × 10″ apart requires a calculated 10.5 lb. per 1000 sq. ft. At 80 cents a pound[3] the cost of material for 1000 sq. ft. would be $8.40 or .84 ct. per sq. ft. ($366 an acre). Ethylene dichloride, using 15-cc. injections, requires a calculated 61 lb. per 1000 sq. ft., which, at the 1939 price of 6.5 ct. per lb.[4] would be only $3.97, or .38 ct. per sq. ft. ($166 an acre). However, at 1941 prices these figures for ethylene dichloride would have to be doubled. Similarly, by calculation, the 1–9 mixture (volume basis) of chloropicrin and ethylene dichloride used at 10 cc. for each injection calls for 5¼ lb. of chloropicrin and 36.6 lb. of ethylene dichloride per 1000 sq. ft. The cost amounted to $6.57 two years ago, but would now be closer to $8.94, or .89 ct. per sq. ft. ($388 an acre). In practice, 10 to 15 per cent should be added to all these figures to care for losses due to evaporation, to extra injections made around the margins, to inaccuracies in calibration of the injectors, to spilling during the many filling operations, and to frequent testing of the injectors.

For comparison, we may cite the costs of steaming, given by Newhall (7) as .65 to 2.0 ct. per sq. ft., depending on the method used and the depth of penetration. Much of the expense of steaming derives from cost of equipment and labor. In these two respects fumigation of soil by volatile liquids has distinct advantages.

It seemed noteworthy to the writers that plots treated in September, with no barriers between them, showed such definite margins the following August. No clearer proof is needed to demonstrate the slow rate of lateral movement of *Heterodera marioni* in sandy soil under greenhouse conditions.

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[3] Price, in 180-pound cylinders, November, 1941.

[4] Price, in 55-gallon drum.

SUMMARY

A simple statistical method of evaluating volatile soil fumigants was used to compare certain nematocides against *Heterodera marioni*.

Excellent control of nematodes was obtained on a spring tomato crop by soil treatments the previous September with the following: (1) Chloropicrin at 10.5 lb. per 1000 sq. ft. (460 lb. per acre) applied at 10-inch intervals, at 2 cc. per injection, 4 to 5 in. deep; (2) A mixture of chloropicrin plus ethylene dichloride, 1–9 at 10 cc. per injection (230 lb. chloropicrin plus 1594 lb. ethylene dichloride per acre).

Less effective control was obtained by 15-cc. injections of straight ethylene dichloride, 2656 lb. per acre. It is concluded that at the recently advanced 1941 price of 12 ct. a pound ethylene dichloride is not recommendable either by itself or mixed with chloropicrin. At sufficiently lower prices its use as a substitute for half of the chloropicrin may be worth considering as an economy measure.

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STRAWBERRY LEAF ROLL, A NEW DISEASE[1]

G. H. BERKELEY[2] AND A. G. PLAKIDAS[3]

(Accepted for publication October 23, 1941)

In June, 1938, Premier strawberry plants with a rather "delicate" appearance and a pronounced rolling of leaves were observed in a plantation near St. Catharines, Ontario. As the symptoms in the field suggested none of the known diseases of strawberries, specimens were brought into the laboratory for further study. When these plants had been observed for some time under greenhouse conditions, it was recalled that in 1935 a Parson's Beauty with similar symptoms had been grafted to a healthy Royal Sovereign and that the disease had been transmitted to the Royal Sovereign and 3 of its runners, but that all the plants had died shortly thereafter. In September, 1940, the junior writer observed a similar disease at Geneva, New York, on seedlings U.S.D.A. No. 1631 and Geneva No. 9270, whilst examining the strawberry-breeding plots there, and notes were compared when the junior writer visited St. Catharines in October, 1940. Since the St. Catharines material had been lost in the meantime, and the junior writer was leaving New York for Louisiana, where the disease does not occur, it was considered advisable to publish this short note on strawberry leaf roll, giving a description of the disease and the limited experimental evidence concerning its nature.

SYMPTOMS

The plant as a whole has a "delicate" appearance, due to small leaves and spindly petioles. However, the most conspicuous symptom is the downward rolling of the leaflets, which is most pronounced in the basal portion (Fig. 1). In extreme cases the rolling is such that the opposite margins of the leaflet touch, or even overlap, thus forming a funnel-shape tube. The leaves on affected plants are pale green, smaller and narrower than normal leaves of the same variety, and the petioles are longer and spindlier. The surface of the leaves is ruffled and rugose and shows irregular chlorotic areas of various sizes. Affected plants are on the whole smaller than normal plants, but there is no pronounced stunting.

TRANSMISSION BY GRAFTING

At St. Catharines, Ont. Under greenhouse conditions 3 of the 4 Premier plants used for grafting made good growth and approached the appearance of healthy plants, though they still maintained a "delicate" type of growth due to spindly petioles and smaller leaves. Runners from these 3 plants were grafted[4] to runners of 3 different clonal plants of *Fragaria virginiana*. In one case the union was not successful and, though it appeared to be successful in the other two cases, the abnormal condition was not transmitted by grafting. The fourth Premier plant made poor growth and produced spindly runners that were difficult to graft; in fact, some of the runners dried up shortly after grafting. Nevertheless, 4 runners from this Premier plant were grafted to runners from different clonal plants of *Fragaria virginiana*, with the result that one graft made on July 18, 1938, was successful in transmitting the diseased condition to one *F. virginiana* plant (Clone No. 21) and its two daughter plants, thus indicating that the disease was of virus type.

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[1] Cooperative project, Science Service, Department of Agriculture, Ottawa, and Louisiana Agricultural Experiment Station. Contribution No. 682 from the Division of Botany and Plant Pathology.

[2] Senior Pathologist-in-charge, Dominion Laboratory of Plant Pathology, St. Catharines, Ontario.

[3] Associate Plant Pathologist, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana. The junior writer's part of the work was done while he was spending his sabbatical year at Cornell University.

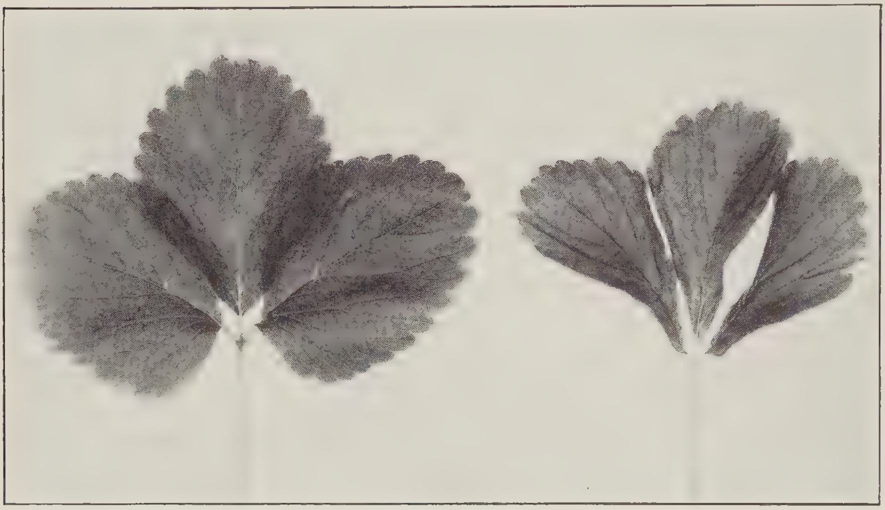

FIG. 1. Strawberry leaf roll. Comparison between healthy and diseased leaves of U.S.D.A. seedling No. 1631. × about ½ natural size.

At Cornell, N. Y. On October 4, 1940, 4 runner grafts were made, 3 between healthy and diseased plants of U.S.D.A. seedling No. 1631, and 1 between diseased and healthy plants of Geneva seedling No. 9270. The grafts were made at the terminal portion of the runners in such manner that the 2 runner-buds could be rooted in the same pot. At this time of the year runner growth was slow and it was doubtful whether the grafts would take. However, 2 of the 4 grafts made union, and in both these cases the disease was transmitted, thus corroborating the results obtained at St. Catharines. The first symptoms appeared 2½ months following grafting. In the other 2 grafts, in which union was not made, no transmission of the disease occurred, even though the healthy and diseased runners were rooted in the same pot and the cut surfaces of their buds came into intimate contact.

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[4] The grafting was done by A. A. Hildebrand, who was carrying on at the time a series of patch grafts in connection with studies on yellow-edge. For description of the grafting technique see "An Investigation on Strawberry Virus Disease in Ontario," by Harris, R. V., and A. A. Hildebrand. Can. Journ. Res., C, 15: 250–280. 1937.

CONCLUSION

Although the number of successful grafts was small, the evidence, nevertheless, indicates that strawberry leaf roll as described is a new, unrecorded virus disease of strawberries.

PHYTOPATHOLOGICAL NOTES

Banded Chlorosis, a Transmissible Disease of Cherry.[1]—In September, 1940, an interesting chlorotic pattern in the leaves of 2 varieties of Japanese flowering cherry was discovered. Further search for these symptoms indicates that they are commonly to be found in the Pacific Northwest on the 3 varieties of *Prunus serrulata* known as Amanogawa, Okochin, and Temari. Hundreds of cases of bud perpetuation were found in nurseries. This led to a further preliminary investigation of the disease, the leaf symptoms of which are rather variable.

There is one constant characteristic of the leaf symptoms. Whatever form the discolored area takes, it is bounded by a chlorotic band, usually about 1 to 2 mm. broad. The band may describe a circle, causing a ring spot or a chain of rings, usually between 2 lateral pinnate veins or along the leaf margin. In other cases more or less perfect "oak leaf" patterns are formed from the midvein and extending out to points on the lateral veins. In other cases a mere sector extending in from the leaf margin may be involved (Fig. 1). The chlorotic areas are whitish or yellowish, sometimes becoming pinkish, in striking contrast to the green of the leaf. The only other apparent effect of the disease on flowering cherries was noted as a dieback of twigs of affected Amanogawa trees 16 or more years old. This dieback does not occur on young trees, and may not be a direct effect of the virus.

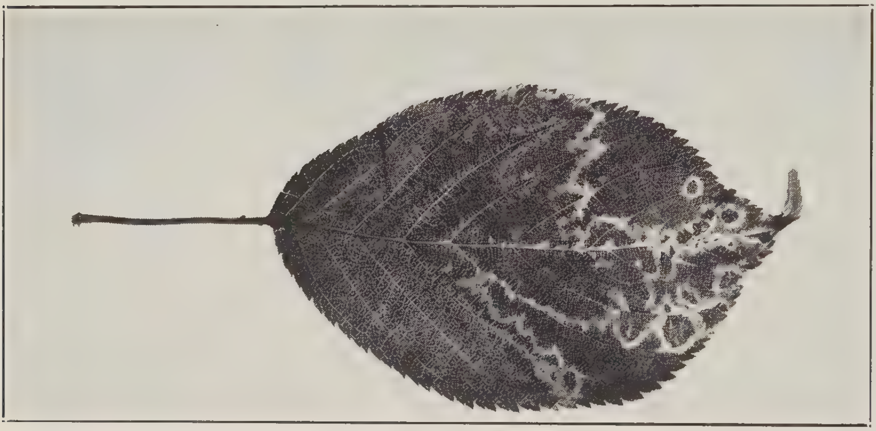

FIG. 1. A leaf of Amanogawa flowering cherry showing typical banded chlorosis.

The first year's experiments with the disease involved the budding of 10 healthy mazzard seedling trees with diseased flowering-cherry buds of the Amanogawa variety. Ten seedlings were budded with healthy Amanogawa checks. The budding was done in September, 1940, and in the late spring of 1941; 9 of the budded trees (1 died) showed symptoms on the leaves of the mazzard stocks. By summer the inoculated trees were dwarfed in comparison with the symptomless check trees. The disease, therefore, is not only transmissible to mazzard stock but also has a detrimental effect, especially on this stock. It was easily transmitted through budding, even though the buds themselves did not "take."

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[1] Published as Technical Paper No. 394, with the approval of the Director of the Oregon Experiment Station. Contribution from the Department of Botany.

Many cases of transmission from flowering cherry to mazzard have been discovered this year in nurseries. Wherever there are mazzard sprouts from stocks supporting diseased Amanogawa tops, these sprouts usually show symptoms of banded chlorosis.

It is possible that dieback in older diseased trees of the Amanogawa variety may be due to the virus in the mazzard roots rather than the influence of the virus in the flowering-cherry cions.

Banded chlorosis, therefore, may be numbered among the transmissible viroses of *Prunus*. Rather elaborate studies of the disease are in progress to determine host range in the genus *Prunus*. As a binomial we suggest the name *Marmor pallidolimbatus* and "Prunus virus 10" in the numerical system.—S. M. ZELLER and J. A. MILBRATH, Oregon State College, Corvallis, Oregon.

Apothecia of Sclerotinia fructicola on Peach in Western Washington.—The occurrence of apothecia of *Sclerotinia fructicola* (Wint.) Rehm. has not been reported on peach in the State of Washington, according to the records in the plant-disease survey file in the Department of Plant Pathology at Pullman.

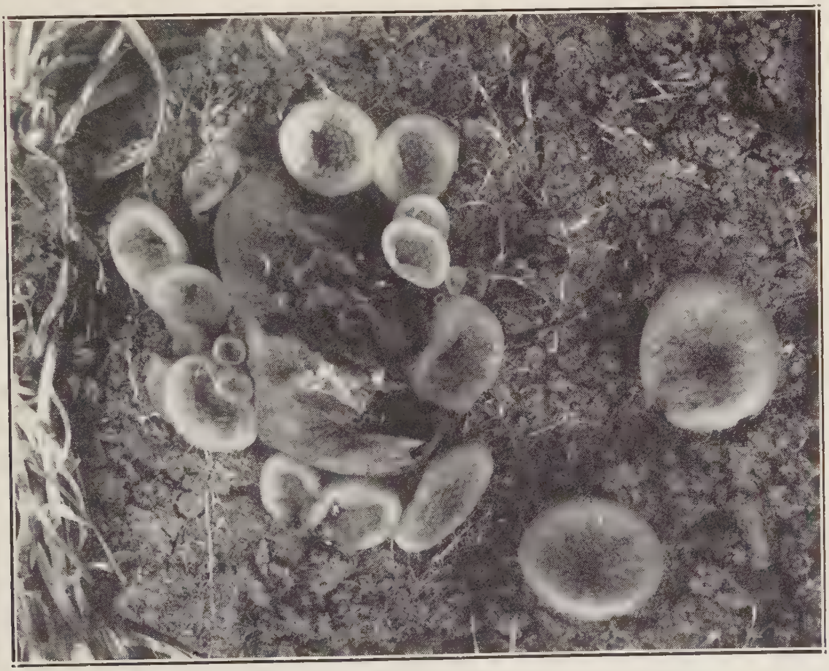

FIG. 1. Apothecia of *Sclerotinia fructicola* developing from a peach mummy.

In western Washington *Sclerotinia fructicola* causes considerable fruit decay of peach during the harvest period.[1] It may live over winter in mummies attached to trees and produce fresh moniliospores the following spring. Apothecia on mummies overwintering on the soil were not observed until the spring of 1941, although surveys in peach orchards had been made each spring for the four previous years. On March 26, 1941, apothecia were found developing from peach mummies beneath a tree in an orchard located in the Lake Shore district near Vancouver, Clark County, Washington (Fig. 1). A survey throughout the rest of that orchard and in other peach orchards of Clark County failed to reveal the presence of additional apothecia on peach mummies. Isolations were made from the apothecia. The cultural characters resembled those obtained from apothecia of *S. fructicola* isolated from Italian prune mummies. Inoculations with pure cultures of the organism caused fruit decay of both peach and Italian prune.—GLENN A. HUBER and KARL BAUR, Western Washington Experiment Station, Puyallup, Washington.

A Simple Technique for Aseptic Handling of Media.—The literature of phytopathology contains descriptions of techniques for measuring and dispensing sterile liquid culture media. Such methods usually call for a certain amount of apparatus of a sort not readily available in many laboratories.

Over a period of several years the writer has developed, and repeatedly used, a very simple method for dispensing sterile media to sterile flasks or adding sterile chemicals to previously sterilized flasks of media. The number of contaminations that have occurred in experiments in which this method has been employed is very small.

The technique is so simple that any person interested in employing it can readily make alterations of details to suit his own special needs. A brief outline follows:

1. Sterilize liquid culture media, vitamins, or other solutions, by filtration or other suitable methods.

2. Prepare empty culture flasks, or flasks of media to receive sterile chemicals, as follows: plug with cotton, make a cap of paper over the cotton (held on by a rubber band), and autoclave.

3. Plug with cotton, wrap in paper, and autoclave, a suitable number of graduate cylinders of suitable size. (50-cc. cylinders have been used extensively by the writer.) Each cylinder may be used safely for at least six to ten measurements.

4. Construct a transfer chamber. Arrange 4 or 6 ring stands, or two or three empty cartons, so as to form the outline of a chamber of the following dimensions: height, 20 inches; depth, 30 inches; and breadth, 30 inches. Cut pieces of cheese cloth, wet with 3 per cent lysol solution, and so hang them from the supports as to provide the chamber with walls and

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[1] Huber, Glenn A., and Karl Baur. Brown rot on stone fruits in western Washington. Phytopath. 31: 718–731. 1941.

ceiling of wet cheese cloth. Sponge with Lysol the table and other exposed surfaces within the chamber. The front curtain must have sufficient slack to allow the worker's arms to perform under it.

5. Place all sterilized equipment, media, etc., in the chamber. Then, with an atomizer (drug store type), thoroughly spray the air of the chamber with a disinfectant solution. This removes any possible contaminating dust.

6. Just before entering the chamber wash the hands and arms with 70 per cent alcohol.

7. The removal of the paper caps from the flasks, or of the paper wrapping from the graduate cylinders, provides sterile, dust-free, cotton-plugged vessels. When the bacterial filter is removed from the filter flask of sterile medium, the lip is wiped with cotton soaked in 70 per cent alcohol. A sterile cotton plug is then transferred from an empty dummy flask to the filter flask, replacing the discarded filter apparatus.

8. Media may now be measured in a graduate cylinder and put into flasks without the use of a flame. Cotton plugs are handled in the usual manner. The plugs being sterile, and the air and the worker's hands comparatively germ-free, it is possible to make repeated measurements and transfers of media with no contamination. For adding 1-cc. portions of sterile solutions to flasks of sterile media, medicine droppers have been calibrated and sterilized in vials.

9. A desk lamp, inside the chamber, is a great aid to visibility through the cheese cloth. Turn out the ceiling lights in the room to reduce light reflection on the outside of the cloth.

The technique suggested is not considered superior to any others previously described in the literature, but may be useful in cases where equipment is very limited or where it is impractical to set up more elaborate apparatus.—J. ARTHUR HERRICK, Kent State University, Kent, Ohio.

A Nonpathogenic Buff-colored Barley Smut.[1]—Buffcolored chlamydospores of certain smut fungi have been found to cause infection on gramineous hosts. To the writer's knowledge, however, the nonpathogenic type has not been described. Campagna[2] reported the occurrence in nature of a white loose smut on wheat. Holton,[3] in studies on hybridization between *Ustilago levis* (Kell. and Sw.) Magn. and *U. avenae* (Pers.) Jens., discovered a buff smut on oats, which originated by mutation in *U. levis.* The chlamydospores were smooth and colorless. Johnson, Rodenhiser, and Lefebvre,[4] in a study to determine the effect of incubation-period temperature on the pathogenicity of *Sorosporium syntherismae* (Pk.) Farl., observed one smutted plant to have buff sori composed of hyaline, glabrous chlamydospores. Moore and Allison,[5] in 1934, found a single head of barley infected with what was described as an albino strain of *Ustilago hordei* (Pers.) Kell. and Sw. The head was almost white, and intermediate between the loose and covered smut type. The spores were colorless, glabrous, and smaller than those of *U. hordei,* and the germination was typical of the sporidium-forming smuts. Two sex groups were identified within the albino strain, monosporidial lines of which were compatible with those of the normal *U. hordei* and *U. medians.*

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[1] Cooperative investigation of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Idaho Agricultural Experiment Station. Published with approval of the Director of the Idaho Station as Research Paper No. 196.

[2] Campagna, E. New white smut collected at Sainte-Anne-de-la Pocatière. Ann. Rept. Que. Soc. Prot. Plants 18: 71–72. 1926.

[3] Holton, C. S. Hybridization and segregation in the oat smuts. Phytopath. 21: 835–842. 1931.

[4] Johnson, H. W., H. A. Rodenhiser, and C. L. Lefebvre. Two types of fall Panicum smut. Jour. Agr. Res. [U. S.] 61: 865–875. 1941.

In September of 1936, the writer commenced a study of the genetics and hybridization between a physiologic race of *Ustilago hordei* and two races of *U. nigra* Tapke. F_1 hybrid chlamydospores were obtained on Odessa barley (C.I. 934)[6] from infection with paired monosporidial lines of *U. hordei* and *U. nigra.* These chlamydospores and those of subsequent generations were collected from individual heads and used to reinoculate seed of the barley varieties, Nepal (C.I. 595), Lion (C.I. 923), and Himalaya (C.I. 1312). On one plant of Nepal there were 2 identical buff smutted heads that contained F_3 chlamydospores. Except for color, these buff smutted heads were identical with those containing the usual black chlamydospores of *U. hordei.*

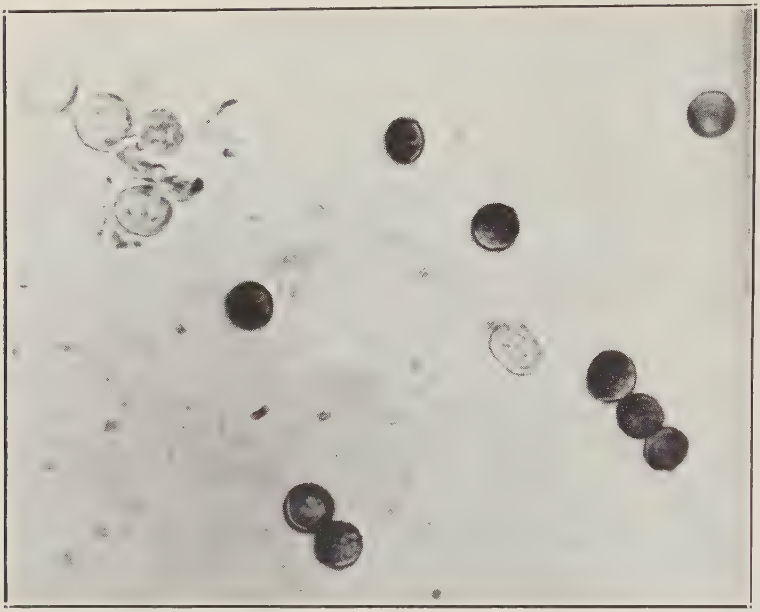

FIG. 1. Photomicrograph showing the contrast in color between chlamydospores of the buff smut of barley and those of the normal *Ustilago hordei.* × 900.

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[5] Moore, M. B., and C. C. Allison. An albino strain of barley smut. (Abstract) Phytopath. **25**: 27–28. 1935.

[6] C.I. refers to accession number of the Division of Cereal Crops and Diseases, B.P.I., U. S. Department of Agriculture.

The spores were hyaline, glabrous, and apparently intermediate in size between *U. hordei* and *U. nigra* (Fig. 1). The sporidia were smaller than the sporidia of either *U. hordei* or *U. nigra,* but more nearly approached the *U. nigra* type; *i.e.,* they were long and narrow and somewhat pointed. Spore germination was irregular. Two or three sporidia instead of the expected 4 sporidia on a promycelium was rather common. The sporidia were difficult to detach from the promycelium. Fusion of sporidia in culture revealed 2 sex groups; however, pathogenicity tests using either chlamydospores or paired monosporidial lines failed to produce smut on Nepal or Odessa barley. This would indicate that the factors governing sex and pathogenicity are different.—WAYNE M. BEVER, College of Agriculture, University of Illinois, Urbana, Ill.

Heterocaryosis and Variability.—Some years ago, a paper[1] was published in which evidence was presented to show that monosporic cultures of *Botrytis cinerea* Pers. may contain genetically different nuclei. With the intent to clarify certain concepts of fungal variability, the writers pointed out that the term heterocaryosis precisely describes such a condition.

It has since become apparent that several writers have used this term in a restricted sense. For example, one author[2] states: ". . . each cell in both the mycelium and conidia of the Fusarium species contains one nucleus. . . . It is, therefore, concluded that heterocaryosis is not responsible for such saltations in these species." Another[3] writes: ". . . it seems very improbable that the induced sectoring in *H. sativum* race 1 was due to heterocaryosis." More recently, others state:[4] ". . . the fact that the vegetative cells are uninucleate seems to eliminate heterocaryosis as a cause of heritable variation in this organism."

From the above citations it appears that these writers either suspected heterocaryosis of being able to cause mutation or thought that such a view had been expressed in one of the earlier papers[1] on the subject. All agree in their conclusions that heterocaryosis is not the cause of mutation, two of them[2,4] appear to reach this conclusion on the basis that the cells of their organisms were uninucleate.

The writer agrees that heterocaryosis is not a cause of mutation. He also agrees that heterocaryosis cannot occur in uninucleate cells. In this connection, however, it is well to remember that at least two factors or mechanisms operate against the possibility of the uninucleate condition being continuous or permanent in any fungus. These two factors, mitosis and anastomosis, operating singly or together are able to produce binucleate, trinucleate and quadrinucleate cells in fungi generally considered to have uninucleate cells. The behavior of some such fungi when they are heterocaryotic has already been described.[5]

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[1] Hansen, H. N., and Ralph E. Smith. The mechanism of variation in imperfect fungi: *Botrytis cinerea.* Phytopath. 22: 953–964. 1932.

[2] Dickinson, Sydney. The nature of saltation in Fusarium and Helminthosporium. Minnesota Agr. Exp. Sta. Tech. Bull. 88. 42 pp. 1932.

[3] Christensen, J. J., and F. R. Davis. Variation in *Helminthosporium sativum* induced by a toxic substance produced by *Bacillus mesentaricus.* Phytopath. 30: 1017–1032. 1940.

[4] Keitt, G. W., and M. H. Langford. *Venturia inaequalis* (Cke.) Wint. I. A groundwork for genetic studies. Amer. Jour. Bot. 28: 805–820. 1941.

What is heterocaryosis? Hansen and Smith[1] state in a footnote that "The term 'heterocaryosis' precisely describes the condition of a cell containing two or more genetically different nuclei. . . ." It was not their intent that the term should or could be restricted as to where such a condition might occur. Heterocaryosis then is a term that exactly describes a *condition;* hence, the term may be used wherever such a condition exists not only in a single cell, but also when it exists in any or all cells of an individual thallus or organism. It may also be used in its adjective form to describe any structure in which the condition occurs. For example: the ascus of any heterothallic fungus is homocaryotic at one time and heterocaryotic at another. *Phoma terrestris* Han. has been isolated from nature only in the heterocaryotic condition.[5] A homocaryotic strain of a species of *Hypomyces* became heterocaryotic in culture by means of a mutation.[6]

How is heterocaryosis initiated? In two ways: (1) by mutation within a plurinucleate entity, and (2) by fusion or anastomoses between cells having genetically unlike nuclei.

How is heterocaryosis related to variability? It has been shown[5] that many fungi occur in nature in a heterocaryotic condition and when such fungi are isolated and worked with in the laboratory they may give the impression of great variability by producing tufts, patches, or sectors differing in appearance from the main part of the culture. In a fungus with multinucleate spores and two kinds of nuclei, variability appears to be even greater when many single spore cultures are made, for among these will be seen several distinct types, two of which are homocaryotic and the rest heterocaryotic. Among the latter may be present several culturally distinct types, because they contain different proportions of the two kinds of nuclei.[5] Heterocaryosis may, in this way, appear to be the cause of variability, whereas in reality, the basic cause of variability and the primary cause of heterocaryosis is mutation.—H. N. HANSEN, Division of Plant Pathology, University of California, Berkeley, California.

On the Cause of the Milo Disease.[1]—The cause of the root, crown, and shoot rot of milo, or Pythium root rot of milo, more commonly known as the "milo disease," according to investigations so far conducted, has been ascribed to *Pythium arrhenomanes* Drechs.[2,3] More recent studies begun by the writer in 1938 show that this sorghum disease is the result of a more complex set of factors than the literature states. The causal factors responsible for the typical symptoms shown by seedling and mature susceptible milo plants and milo hybrids in the greenhouse and field cannot be attributed to *P. arrhenomanes* alone.

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[5] Hansen, H. N. The dual phenomenon in imperfect fungi. Mycologia **30**: 442–455. 1938.

[6] Hansen, H. N., and W. C. Snyder. The origin and inheritance of *M* types in Hypomyces. Phytopath. **30**: 787. 1940.

[1] Contribution No. 430, Dept. of Botany, Kansas Agricultural Experiment Station.

[2] Elliott, Charlotte, L. E. Melchers, C. L. Lefebvre, and F. A. Wagner. Pythium root rot of milo. Jour. Agr. Res. [U. S.] **54**: 797–834. 1937.

[3] Kendrick, J. B., and F. N. Briggs. Pythium root rot of milo and the development of resistant varieties. Calif. Agr. Exp. Stat. Bull. 629. 1939.

When susceptible varieties and resistant selections of milos are grown in naturally infested soil in the greenhouse or field, susceptible varieties show very definite symptoms, followed by the death of the plants, while resistant selections and varieties remain alive and show marked resistance.[2] Recent investigations by the writer show that typical symptoms of the milo disease are not produced in the seedling stage in the greenhouse or in older plants in the field when grown in sterile or nonsterile soil containing pure cultures of *P. arrhenomanes.* Furthermore, it has been learned that this pathogen is equally pathogenic to susceptible milos and to the strains of milo and other common sorghums known to be resistant to the milo disease. Certain organisms, environmental conditions, or extenuating factors other than those studied in the investigations of this disease[2,3] are etiologically involved in the problem.

It is realized that the foregoing statements from an etiological point of view are largely negative, yet the facts represent information obtained in a study of the causal factors concerned in this disease. More detailed results of the present studies will appear in another paper. D. B. Creager and C. M. Slagg have assisted in these investigations.—L. E. Melchers, Dept. of Botany, Kansas Agricultural Experiment Station, Manhattan, Kansas.

Rhizoctonia Infection of Cotton and Symptoms Accompanying the Disease in Plants beyond the Seedling Stage.—Rhizoctonia (*Corticum vagum* Berk. and Curt.) is one of the causes of damping off of cotton seedlings. The attack usually occurs on seedlings in early planted cotton prior to the formation of true leaves. The pathogen invades the stem immediately below the soil line and causes the formation of dark to reddish-brown cankers. In severe attacks the cankers may encircle the stem and penetrate so deeply that the seedlings fall over and die. An unusual stage of the disease was observed in the Louisiana Delta in 1940 and 1941. The affected plants were in the early flowering stage, from 7 to 14 inches high. It was found that many of the plants were almost devoid of root systems especially lateral roots and were easily uprooted (Fig. 1, A). They were growing in a more or less prostrate condition and had few fruiting branches. Stems of affected plants had deep-seated cankers both above and below the soil line. Many showed typical "constrictions" that almost severed the stems a short distance below the surface of the soil (Fig. 1, B). Tissue cultures of infected plants yielded, in approximately 90 per cent of the platings, the Rhizoctonia fungus. It appears, therefore, that when cold, wet weather continues through late spring, the disease may persist and cause considerable injury to older cotton plants.—David C. Neal, Bureau of Plant Industry, U. S.

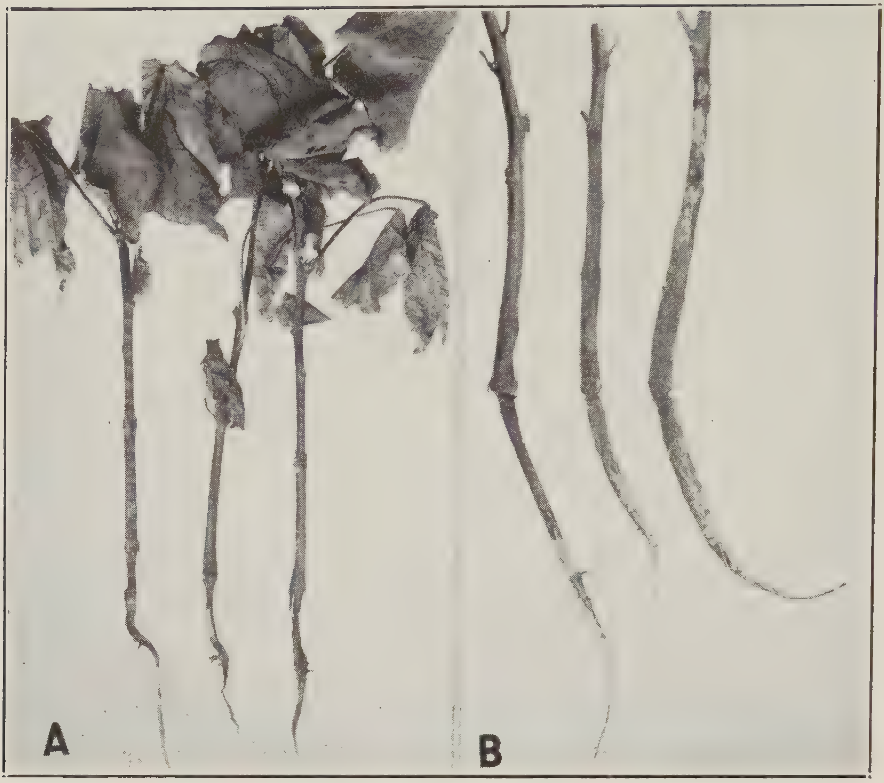

FIG. 1. Unusual symptoms of Rhizoctonia (*Corticium vagum*) infection of cotton. A. Delfos 425 plants infected with Rhizoctonia and approaching flowering stage. Note poorly developed root system, scarcity of lateral roots, and deep cankers on stems below soil line. B. Delfos 425 plants. Same age as those shown in A. Note cankers both above and below soil line and typical "constrictions" on stems. Plants collected and photographed on June 25, 1941.

Department of Agriculture and Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana.

Olive Anthracnose in the United States.—A disease of the olive fruits was found on the Mission variety in December, 1941, on the campus of the University of California in Berkeley. The disease appeared to be quite similar to the olive anthracnose described in 1899 by d'Almeida[1] in Portugal. Initial symptoms of the disease consist of small brown spots on the ripe, as well as green, surfaces of unripe fruits. The lesions increase gradually in size, becoming irregular and depressed in shape. Later, the surface of the spots becomes brick-red in general appearance, owing to sporulation of the causal fungus, and in some cases turns black.

The olive anthracnose has been observed in France,[2] Spain,[3] Japan,[4]

[1] Almeida, J. V. d'. La "Gaffa" des olives en Portugal. Bull. Soc. Myc. France **15**: 90–94. 1899.

[2] Griffon, E., and A. Maublanc. Notes de pathologie végétale et animale. Bull. Soc. Myc. France **17**: 469. 1911.

Russia,[5] Greece,[6,7] and Uruguay.[8] Only in Portugal and in Greece has the disease caused important damage, reducing the quality of the fruits. In California also, it may become serious, particularly on olives used for oil production.

The fungus was readily isolated on potato-dextrose agar in Petri dishes by tissue plantings from the fruits and by direct transfer of the spores from the lesions. The organism has been identified as *Gloesporium olivarum* Alm. Like many other imperfect fungi, this fungus exhibits the *dual phenomenon*,[9] that is, when single-spore cultures were made 2 types appeared: the mycelial

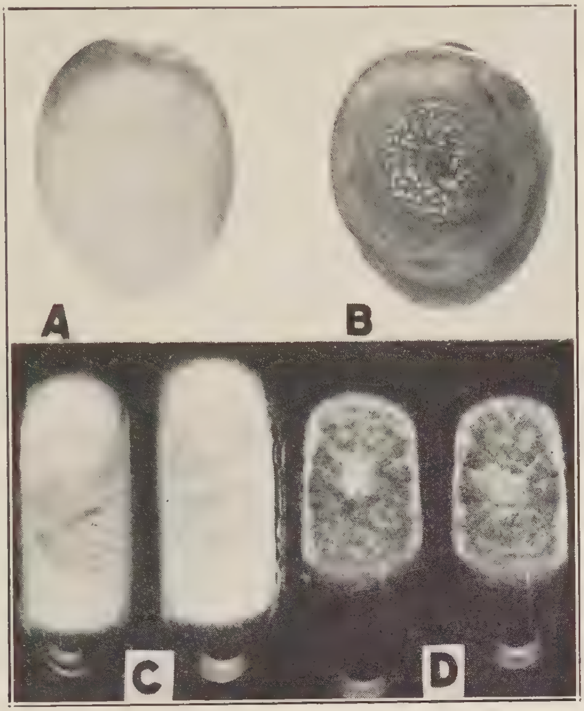

FIG. 1. A. Healthy fruit. B. Inoculated fruit. C. The mycelial type. D. The conidial type.

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[3] Navarro and Nonell. Epifitias mas importantes del Olivo en España. VII Congres Int. Oleiculture. (Seville.) 311 pp. 1924.

[4] Hemmi, T., and S. Kurata. Contributions to the knowledge of anthracnose of plants. Jour. Soc. Trop. Agr. Taiwan. **6**: 573–583. 1935.

[5] Nagorny, P. I., and E. M. Eristavi. A brief survey of plant diseases in Abkhasia in 1928. Publications Agr. Exp. Sta. of Abkhasia 38. 28 pp. 1929. *In* The Rev. App. Myc. 9: 326. 1930.

[6] Petri, L. Azione tossica dell' arsenito sodico sofra le spore del *Gloesporium olivarum* Alm. Boll. R. Staz. Pat. Veg. Ann. **10**: 359–361. 1930.

[7] Sarejanni, J. A. Catalogue commenté des champignons rencontrés sur les plantes cultivées en Grèce. Int. Phytopath. Benaki. (Athens) Ann. 3: 60. 1939.

[8] Acosta, D. R. Investigaciones fitopatologicas. Min. Industrias. Direc. Agron. Publ. Mensual 4 (12): 1–18. 1932.

[9] Hansen, H. N. The dual phenomenon in imperfect fungi. Mycologia **30**: 442–455. 1938.

type producing abundant mycelium and few conidia, and the conidial type producing many conidia and less mycelium (Fig. 1, C, D).

Inoculations of wounded fruits of the Mission variety were made separately with spore suspensions of the conidial and of the mycelial types of the fungus. Symptoms were apparent after 36 hours, and 1 week later the acervuli appeared (Fig. 1, A, B). The 2 types that were reisolated proved to be identical with those obtained from the original cultures.

No great difference in virulence between the conidial and mycelial types could be detected as a result of these tests; both were pathogenic to the Mission variety.

A careful search of the literature indicates that this fungus has not previously been reported in the United States. Fruit specimens of the disease have been deposited in the herbarium of the Department of Botany, University of California, Berkeley.—RAFAEL E. PONTIS, Research Fellow of the Asociacion Argentina para el Progreso de las Ciencias, and H. N. HANSEN, Division of Plant Pathology, University of California, Berkeley.

Mycological Nomenclature.—The International Rules of Botanical Nomenclature are intended to give fixity to names, to avoid error, ambiguity, confusion, and the useless creation of names (Art. 4).

American Plant Pathologists have followed the spirit of these Rules. I hope they, and other mycologists, will give formal consideration to two changes I hereby propose, and that are being considered also in Britain:

1. That, *whenever necessary among Fungi, the conservation of specific names, firmly established in the literature by wide use over many years, be legalized.* For example, the specific epithets in the names *Tilletia tritici, T. laevis, Ustilago levis* and *Rhizopus nigricans* are illegitimate under the existing rules; their use will continue in any event, and should be legalized. Also, about a score of pre-Friesian names of Powdery Mildews used by Salmon and all subsequent mycologists could be badly confused by anyone who tried to interpret the Rules to the letter.

2. That Article 57 be rewritten somewhat as follows: *Among Ascomycetes and Basidiomycetes (but not Phycomycetes) with pleomorphic life-cycles, the first valid binary name applied to the perfect state of a species takes precedence. The names of imperfect states can still be used, and should be used if ambiguity might follow the use of the name of the perfect state.*

Thus, for example, mycologists now use, and it should be made legitimate for them to use, *Cladosporium herbarum* Fr. despite the fact that its perfect stage *Sphaerella Tulasnei* Jancz. has been found and verified. It may be misleading to cite the name of a perfect stage from a country where only the imperfect stage is known.

Those who may think Phycomycetes should bear the names (and, of course, the authorities) used when the perfect state (oospore or zygospore) of each species was first described should try to provide author citations for

Phytophthora infestans, other Peronosporales, and the zygomycetes. Article 5, in my opinion, excludes Art. 57 from applying to Phycomycetes; for the consequences of such application are more than doubtful, and it is established custom to use the first valid name applied, whether to imperfect or perfect state.—G. R. BISBY, Imperial Mycological Institute, Kew, Surrey, England.

BOOK REVIEWS

HOLTON, CHARLES STEWART, and FREDERICK DEFOREST HEALD. *Bunt or Stinking Smut of Wheat.* 211 p., 21 figs., Burgess Publishing Co., Minneapolis, Minnesota (U.S.A.) 1941. $3.25.

In this book the authors have summarized and briefly discussed most of the important investigations that have been reported on bunt and its causal organisms. The authors have fulfilled the purpose which they claim for the volume "... to make available a single publication that will serve as a source of general information on the present status of the bunt problem in its various aspects." The reviewer feels that it is fortunate that two men as well qualified for analyzing work with bunt as Holton and Heald undertook to bring together this information from its diverse sources and give us the benefit of their interpretations. They have produced a reference work with an almost complete bibliography and a resumé of what has been accomplished by the investigations which have been made with bunt. The book is in mimeoprint and is illustrated with 21 plates of well chosen material.

The book is divided into 11 chapters, each dealing with a separate phase of the bunt problem. The chapter headings in abbreviated form are: I. Introduction, II. Economic Importance, II. Species Distinction, Spore Germination, and Artificial Culturing, IV. Host Range, V. Factors Affecting Infection and Development, VI. Effect of Bunt on Morphology and Physiology of the Wheat Plant, VII. Physiologic Specialization, VIII. Cytology, IX. Heterothallism, Hybridization and Species Assocition, X. Varietal Reaction and Genetics of Resistance, and XI. Control Measures. In each chapter the authors have abstracted and discussed the papers they considered as giving the most important contributions to that particular phase of the problem. After reviewing the literature they have, in most chapters, summarized the results and pointed out the accomplishments, as well as the instances where fundamental information is lacking. These summaries and critiques add much to the value of the book. For each chapter a bibliography is given, and these include many references not cited in the text. There are more than 1100 references listed, 635 of these in Chapter XI on Control Measures.

A summary of the seed treatments that appeared to have been most effective and practicable in controlling bunt would have added materially to Chapter XI. Only a few errors are evident in the printing. Chapters IV, VII, and X should be of particular interest to plant breeders, as well as plant pathologists.—R. H. BAMBERG, Bureau of Plant Industry, U. S. Dept. of Agriculture.

GUILLIERMOND, ALEXANDRE. *The Cytoplasm of the Plant Cell.* Chronica Botanica Co., Waltham, Mass., 1941, 241 p., 152 figs.

Studies on damaged and destroyed cells, important as they are, reveal little of the nature of cellular organization, in its relation to metabolism, hence the limitations of physiological researches conducted on tissue extracts, *breis*. . . . One of the most variable respiratory systems of the cell, that which has been studied by Warburg, Keilin, and recently by Woods and DuBuy, seems closely bound to cell structure and greatly affected by alterations of that structure.

As the chemist has to know of the structure of molecules, so the biochemist has to know how molecules link up into the cellular structure, as perceptible through cytological observation.

To correlate the expression of disease with unbalanced cell metabolism, the pathologist has to interpret such changes in the cell structure as he can detect through direct microscopic observation of living tissues, or through the use of cytological or cytophysiological methods; from cytological data he can obtain information on the shifting of the balance between the various enzymatic systems on the interplay of which cell metabolism depends.

Guilliermond's exposition of the physical properties of the cytoplasm, of its chemical constituents and physico-chemical constitution, of the plastids and mitochondria and of their relationship, of the vacuoles, and lastly, of fatty degeneration and cytoplasmic alterations, lead us, through the use of a "convergent technique" to "seek the relationship between morphological structure and the physiological activity of the cell." The "cytologist must become both a chemist and a physicist," he "must at the same time remain a morphologist."—J. DUFRENOY, Louisiana State University, Baton Rouge, Louisiana.

REPORT OF THE 1942 ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 1942 annual meeting of the Southern Division of The American Phytopathological Society was held in part as a section at the meeting of the Association of Southern Agricultural Workers, February 4, 5, 6, in Memphis, Tennessee. A joint session was held with the Agronomy Section on diseases of forage crops. About 50 plant pathologists attended the meeting, and approximately 12 formal papers were given. The fact that the meetings of The American Phytopathological Society were held in Dallas, Texas, in late December, 1941, explains the limited number of papers presented at Memphis in February 1942. A short business session was held on the morning of February 5, when the following officers were elected.

President, S. G. Lehman
Vice-President, W. H. Tharp
Counselor, A. G. Plakidas

Titles and abstracts of papers presented at the meeting follow.

I. L. Forbes
Secretary-Treasurer

Breeding Tobacco for Black-root-rot Resistance. **Henderson, R. G.** Breeding tobacco for resistance to black root-rot (*Thielaviopsis basicola*) has been carried on for the past 8 years. Crosses have been made between a root-rot-resistant variety of Turkish (Xanthia) tobacco and susceptible flue-cured and dark fire-cured varieties. The F_1 plants were larger than the Turkish parent but resembled it in most other respects. They were resistant to root rot, but the degree of resistance was not determined. The F_2 plants were extremely variable in leaf size and shape, petiole length, number of leaves, etc.; otherwise, they were intermediate in appearance between the two parents with closer resemblance to the Turkish parent. There was a noticeable difference in degree of resistance in the F_2 population. Selections in the F_2 and F_3 populations gave lines highly resistant to root rot. The best of these lines, however, closely resembled the Turkish parent, although some advance was made in obtaining a resistant plant of desirable type. Selections from the F_2, F_3, and later generations were backcrossed with the susceptible parent, but resistance was maintained more consistently at a high level when F_3, or later generations, were used. Selections in the progeny of the backcrosses gave resistant plants resembling the susceptible parent in general appearance. The leaves of the most resistant lines, however, were still small. A second backcross was required before plants with full-sized leaves were obtained.

In 1941 several resistant hybrids of the flue-cured type were tested on soil infested with *Thielaviopsis basicola*. One of these, No. 38, was highly resistant to root rot and appeared to have other desirable characteristics. The average height of the plants of this hybrid on August 11 was 74.4 in., while that of the adjacent check (Cash) plants was 37.5 in. Hybrid No. 38 also was included in the variety test at Chatham, Virginia, where root rot was not a factor. The hybrid produced 1264 lb. of cured tobacco per acre, valued at $420.80, as compared to the check (Yellow Mammoth), which yielded 1072 lb. per A., valued at $328.80.

The growth rate of the most resistant hybrids, as determined by height measurements, progressed at a uniform rate from the time the plants were well established in the field until they reached full height; whereas that of susceptible plants was slower during the first 2 months of the growing season than during the last month, indicating that the susceptible plants were stunted by root rot during the cooler part of the season.

Studies on Soil Sterilization with Urea and Calcium Cyanamid. **Henderson, R. G.** Heavy urea and calcium cyanamid applications added to tobacco plant-bed soil have given promising results in weed control. These chemicals produce marked changes in the physical structure, ammonia- and nitrate-nitrogen content, microbial population, pH, and O R potential of the soil. The time required for the urea and calcium cyanamid to be completely ammonified in the soil varies with the soil type and certain environmental factors. Manure and blackstrap molasses, applied with the urea, appear to increase nitrification. The ammonia concentration also apparently decreases more rapidly in plots receiving manure and blackstrap molasses. In an experiment conducted in the greenhouse on Granville type soil, about 60 per cent of the urea-nitrogen applied escaped from the soil as ammonia during a period of 5 months. Ground wheat straw, added to the soil with the urea, reduced this loss to about 33 per cent. The loss of ammonia-nitrogen from calcium cyanamid-treated soil in the same test was less than 9 per cent without straw and only 2.4 per cent with straw. Platinum electrodes were buried in treated and nontreated soil in the greenhouse and, at frequent intervals, millivolt readings were taken from them with a calomel-half cell as a reference. While these readings may not represent the true Eh of the soil, they indicate the extreme change brought about by the heavy application of urea and calcium cyanamid. The millivolt reading dropped from more than 400 to about 50 following the treatment and rose very slowly until at the end of 6 months it was approximately 200. Straw added to the calcium cyanamid caused the reading, following the initial depression, to rise above 300 mv. at the end of the first month; but there was a gradual decline after that time, until it approximately equalled that without straw at the end of 6 months. The drop in E.M.F., due to the rise in pH, would be expected not to exceed 180 mv. On this basis it is evident that these treatments brought about a substantial change in the O R potential.

Oospore Production in Cabbage Seedlings by Peronospora parasitica. LEBEAU, F. J., AND J. A. PINCKARD. Oospores of *P. parasitica* proved abundant in the cotyledons and sparse in the true leaves of field-grown cabbage seedlings in south-central Mississippi. Oospore formation also appeared related to moisture, temperature, and light intensity, since the spores were most frequently observed in the tissues of seedlings in dense stands on the south side of wood-inclosed growing-frames during November and December. Most abundant field production of oospores occurred during rainy periods, which permitted fungal assimilation of food materials from invaded tissues. Dry, bright weather appeared to interrupt oospore formation because of desiccation of invaded tissues. Greenhouse-grown cabbage seedlings surrounded with cheesecloth produced abundant oospores approximately 15 days after inoculation, provided water was continuously atomized into the air above the plants. In view of these observations, the writers conclude that the life cycle of the cabbage downy-mildew fungus is completed with the formation of oospores, largely in the cotyledons, and that over-summering of the fungus is probably by means of oospores rather than by perennial mycelium in weed hosts.

Cotton-seed Treatment with Dust Preparations Containing Hormones Alone and in Combination with Ceresan and Spergon. LEHMAN, S. G. Cotton seed, before planting, was dusted in the field with two hormone preparations, one containing indolbutyricacid, the other potassium naphthalene acetate. Each hormone dust was used alone and in combination with Spergon in 2 tests and with 2 per cent Ceresan in 2 tests. The application rate or chemical seed ratio by weight was 1 to 113,000 for hormones, 1 to 480 for Spergon, and 1 to 160 for Ceresan. Each treatment was planted in a plot of 1 or 3 50-ft. rows in each replication, four replications being planted in each test. Neither hormone produced any observable effect on the number of seedlings emerged nor on the yield of seed cotton. Spergon increased the number of seedlings 11 per cent in one test and 22 per cent in another, both increases being highly significant statistically. Yield of seed cotton increased 6.2 per cent with Spergon in the one test for which yield records were kept. Ceresan gave no significant increase in number of seedlings emerged nor in yield. The tests with Spergon and Ceresan were planted at different dates, in different locations and with different seed varieties.

Results from the B-2 Regional Test with Reginned and Acid-delinted Cotton Seed. LEHMAN, S. G., D. M. SIMPSON, C. H. ROGERS, J. A. PINCKARD, AND C. H. ARNDT. The purpose of this test was to note the effect of removing the fuzzy or short lint from cotton seed before planting. Sublots, treatments, and per cent of lint on the seed were as follows: F, fuzzy (natural) seed, 14.9 per cent lint; R1, reginned, light cut, 8.1 per cent lint; R2, reginned, medium cut, 5.9 per cent lint; R3, reginned, heavy cut, 4.0 per cent lint; A, delinted with sulphuric acid, no lint; AS, delinted with sulphuric acid and subsequently scarified. Following the primary treatments each sublot was divided into 2 aliquots, one of which was dusted with 5 per cent ethyl mercury phosphate dust (New Improved Ceresan) and the other left undusted. Six plantings of all sublots were made in North Carolina, 2 in Tennessee, 1 in Mississippi and 1 in Texas. Three plantings of the Ceresan-treated aliquots were made in South Carolina. In the 6 plantings in North Carolina, the mean percentage increase in number of seedlings at the last count before thinning for each primary treatment above the natural fuzzy sublot was as follows: R1, 13; R2, 22; R3, 23; A, 53; and AS, 48 per cent. Increase for Ceresan dusting was 13 per cent. For the 10 plantings in N. C., Tenn., Miss., and Texas the corresponding percentage increases were as follows: R1, 8; R2, 14; R3, 16; A, 41; and As, 35 per cent; and for Ceresan dusting, 16 per cent. For the 3 plantings in South Carolina, where only the Ceresan dusted aliquots were used, the following percentage increases were obtained; R1, 64; R2, 51; R3, 47; and A, 61 per cent. All of the increases reported above were highly significant statistically. All reginned and all acid-delinted sublots emerged more rapidly than the sublots with the normal amount of lint.

Seed-treatment Studies of Vegetable and Ornamental Plants. PERSON, L. H. AND S. J. P. CHILTON. Tests were made of cuprocide, red copper oxide, yellow copper oxide, zinc oxide, Vasco 4, Ceresan, and Semesan as seed treatments, and yellow copper oxide, zinc oxide, Vasco 4, Leafox 200, formaldehyde dust, and sand as soil treatments for tomatoes, peppers, and eggplants. Seeds treated with the copper dusts gave the best results over a period of 3 years. Semesan and Ceresan were sometimes toxic, and the zinc compounds gave erratic results at times when used on the seed. The copper dusts in water and the zinc compounds dusted on the surface were of equal value as soil treatments. Formaldehyde dust as a soil treatment was of little value. Sand reduced emergence approximately 10 per cent. With tomatoes and peppers, seed treatment with cuprocide, Merck's red copper oxide, or yellow copper oxide was usually sufficient to obtain good stands without soil treatment. With eggplants, seed treatment with the copper dusts, and soil treatment, either with a water suspension of the copper compounds or a covering of one of the zinc compounds given above, was necessary to insure good stands.

Cuprocide, yellow copper oxide, Vasco 4, Leafox 200a, 1286a, 1286ccc, Spergon, Barbak C, Ceresan, New Improved Ceresan, and New Improved Semesan, Jr., were tested on the seed of various ornamental plants in the greenhouse. The mercury dusts were toxic where applied full strength. Yellow copper oxide gave the best results of all compounds tried on centaurea, zinnia, cosmos, calendula, and pansies.

The Effect of Depth of Planting on Fuzzy and Acid-delinted Cotton Seed. RAY, W. WINFIELD. Field experiments were made with Ceresan-treated fuzzy and acid-delinted seed in which 4 depths of planting, 1, 1¾, 2½, and 3¼ inches, at 2 rates and 2 dates were employed. The data for both stands and yields were analyzed by the analysis-of-variance method. The delinted seed proved superior statistically to the fuzzy seed at the 4 depths of planting. No significant differences existed between the 2 shallower depths of planting for either of the 2 seed types, but both of the shallower depths of planting gave stands and yields statistically superior to those of the 2 deeper plantings. It is concluded that the depth of planting normally employed for fuzzy seed is favorable for acid-delinted seed and that the acid-delinted seed gives stands and yields superior to those from fuzzy seed.

Results of Seed Treatment Tests on Peanuts. SHAW, LUTHER. Nineteen chemical dusts were extensively tested in the summer of 1941 to determine their effects on emergence of peanuts when seed-dusted before planting. Results of 3 suitably replicated tests, conducted in the field, show an average emergence of 23.2 per cent for the nontreated plots. One organic sulphur and 2 organic mercury materials consistently gave the greatest emergence, averaging 65.5 per cent for the organic sulphur, and 63.0 and 60.4 per cent for the mercury materials. Spergon, and a 20–80 mixture of lime and sulphur resulted in emergences of 59.4 and 52.9 per cent, respectively. Eight copper compounds were used, the copper content of each dust having been adjusted to 10 per cent with talc as a diluent. All of these materials, excepting one, gave increased emergences of medium rank. Most of the other materials tested gave small increases in emergence. Observations and preliminary experiments indicated that the increases in emergence obtained from seed treatment resulted from prevention of seed decay prior to germination. It was also evident that most of this decay took place during the first 3 to 4 days after planting.

Results of Preliminary Experiments on the Control of Root Diseases of the Peach. SHAW, LUTHER. Results of preliminary experiments conducted at 2 locations in North Carolina in the 1941 season indicate that certain chemical soil treatments offer promising aid in replanting old orchard sites. One of the locations involved an area planted to Elberta peaches for 4 years, and the other an area planted to the same variety for about 20 years. An examination of trees pulled prior to undertaking the experiment revealed the presence of root knot, crown gall, and root rots at both locations, the rots being prominent at the older site only. Bands of soil 6 feet wide, centering on the tree row and extending through 5 old tree sites received varying rates of the following 10 treatments: Chloropicrin, Paradichlorobenzene, benzol, sulphur, cyanamide, urea, basic slag, nitrate of soda, potash, calcium hypochlorite, Spergon, and Spergon No. 98 special. About 2 months after treatment all plots were planted with Belle of Georgia peach trees on native root stock. Two additional nontreated plots were planted with the same variety as controls, and one with the same variety on Shalil root stock. Results of these tests show: (1) Shalil root stock was practically immune from root knot, but was susceptible to crown gall; (2) urea gave excellent disease control at both locations, but severely stunted the trees; (3) chloropicrin resulted in excellent tree survival and vigor at both locations, but gave only moderate root-knot control in the younger orchard site; (4) the remaining treatments gave little to no improvements in disease control or tree survival over that of the controls.

Further Studies on the Reaction of Commercial Cotton Varieties to Root-knot Nematode. SMITH, A. L. Reaction to root-knot of commercial varieties studied in 1941 confirm results previously reported (Phytopath. 31: 1099–1107, 1941). A system is described for expressing root-knot susceptibility in terms of a single figure, the "root-knot index." The range of values varies from 0 to 100, the latter term representing complete susceptibility. This system facilitates comparisons and permits statistical analysis of the data. A comparison of 25 commercial varieties shows Coker's 4 in 1–4 and Coker's 4 in 1–5 to be superior to others in root-knot resistance. Inbred lines selected from commercial varieties varied widely in resistance and indicate possibilities for improving root-knot resistance. Additional evidence was obtained suggesting that root-knot resistance is inherited recessively.

Root-knot Resistance of Five Soybean Varieties. TAYLOR, A. L. A method for testing resistance of plants to the root-knot nematode, *Heterodera marioni*, was devised and tested in 4 experiments at 3 locations on the Laredo, Biloxi, Ototan, Clemson, and Georgian varieties of soybeans. Irregularities in the results at the different locations suggest that root-knot resistance varies with ecological conditions. None of the tested varieties of soybeans was found resistant enough to warrant its recommendation for use in nematode-reducing rotations.

Chemical Control of Root Knot. TAYLOR, A. L. The root-knot nematode, *Heterodera marioni*, can be controlled in soil in the field or greenhouse by the use of chloropicrin, ethylene dichloride, carbon bisulphide or methyl bromide. All except methyl bromide are applied by injecting measured amounts into the soil at regular intervals. Methyl bromide is applied by releasing the gas under an air tight soil cover. Costs per acre, including an allowance for application, are: Chloropicrin, \$180 to \$360; ethylene dichloride, \$340 to \$360; carbon bisulphide, \$70 to \$90; methyl bromide, \$150 to \$200. Carbon bisulphide cannot be generally recommended because of its extreme fire and explosion hazard. Where crops are planted in widely spaced rows or hills, the "spot treatment" technique can be applied with chloropicrin at costs ranging from \$8 to \$10 per acre for watermelons to \$35 to \$40 per acre for tomatoes. Methyl bromide is particularly effective for treating potting soil, complete control of root knot being obtained with $\frac{1}{8}$ ml. per cu. ft. and a cost of less than 1 cent per cu. yd. Ethyl mercury iodide also may be used for control of root knot on potting soil high in organic matter, but appears to be toxic to some species of plants.

Distribution of the Root-knot Nematode in High Ridge Plantings of Potatoes and Tomatoes. THORNE, GERALD. The root-knot nematode, *Heterodera marioni*, has a very definite behavior in the potato fields of western Nevada, where production is by irrigation. The water is applied through deep furrows between the rows. Most of the tubers are produced within ridges formed by "hilling up" the plants as the furrows are dug.

Irrigation water applied to *level* fields stands deep in the furrows and the soil is saturated, giving the nematodes every opportunity to reach plants, with the result that practically every tuber is heavily infected and unfit for use. Water flowing rapidly through the furrows of *sloping* fields rises in the ridges only by capillary action and the soil does not become saturated. Nemic activity is thereby restricted to the lower portions of the ridges, and tubers from the top 4 or 5 inches are generally clean. Below these uninfected tubers a few nematodes appear and the numbers increase with the depth until, near the water level in the furrows, the tubers are severely infected.

Similar conditions were observed in 2 California tomato fields, one near Santa Ana, the other near Modesto. In both instances frequent, shallow irrigation between high ridges produced good crops. Downward growing roots were soon clubbed and deformed by root knot, but those growing laterally made a remarkable growth, often extending 6 to 10 ft. along the ridges with little or no infection. The owner of the Santa Ana field stated that filling the furrows with water just once would destroy the crop within a week.

Moisture appears to be the principal underlying factor in producing the above modes of root-knot infection, since temperatures and soil types were very different in the 2 widely separated localities observed.

Populations of Root-knot Nematode Larvae in Two Kern County, California, Fields. THORNE, GERALD, MERLIN W. ALLEN, JAMES HARE, and MARK A. LINDSAY. A preliminary survey of Kern County, California, in the summer of 1941, showed upwards of 75,000 acres of land infested with the root-knot nematode, *Heterodera marioni*. Two severely infested fields were selected for study, one near Arvin, the other near Shafter. Soil samples were taken in 6-inch columnar sections to a depth of 5 feet and examined for root-knot larvae by the Cobb sifting and gravity methods. Great variation was found in the numbers of larvae in the various sections and between the total samples. Distribution of larvae appears determinable by location of roots of cotton and alfalfa grown in the fields for 3 preceding years. Highest populations of individual samples varied from the 7- to 12- to the 55- to 60-inch sections, usually being in, or below, the 19- to 24-inch section. Most samples failed to reach the extreme depths occupied by the larvae, in some instances the maximum population was in the 55- to 60-inch section, indicating that larvae doubtless would have been found deeper had the samples been taken.

Young roots of alfalfa, following cotton, were rarely attacked, although they were closely associated with thousands of larvae. The reason for this failure to make a ready transfer of hosts is problematical but it may be due to the existence of 2 distinct strains or races of *H. marioni* or to the host preference development when either crop is grown continuously for a number of years.

These studies emphasized the futility of chemical treatment of the soil following alfalfa or cotton in this locality, because of the extreme depths at which a major portion of the nematode population is located. Added evidence is given to the necessity of evaluating host plants in a given locality when planning crop rotations for nemic control.

Cotton-seed Treatments and Angular-leaf-spot Control. YOUNG, V. H. A study involving the Acala variety of cotton was made to determine the effects of sulphuric acid delinting of cotton seed and of flotation grading of the delinted seed into ''floaters'' and ''sinkers,'' combined with the use of New Improved Ceresan on incidence of cotton diseases, percentages of emergence and yields of seed cotton. The following six treatments were employed: Fuzzy seed, nontreated (check); fuzzy seed treated with Ceresan; acid-delinted ''floaters'' nontreated; acid-delinted ''floaters'' treated with Ceresan; acid-delinted ''sinkers'' nontreated; acid-delinted ''sinkers'' treated with Ceresan. All treatments greatly reduced the incidence of seedling diseases in comparison to the fuzzy nontreated checks, but no other treatment was superior to fuzzy seed treated with Ceresan in that respect. Counts made September 1 showed 75 per cent of plants in nontreated check plots affected with angular leaf spot (*Bacterium malvacearum*) compared to 13 to 35 per cent in other plots. Emergence was little affected by any of the treatments employed. Flotation grading in this particular case failed to increase the percentage of emergence. Yields for all plots planted with treated seed were 31 to 48 per cent greater than for plots planted to fuzzy nontreated seed, but differences between treatments were not statistically significant. Data were analyzed statistically by the analysis-of-variance method. Under the conditions of this experiment, all treatments gave highly significant yield increases over nontreated checks and highly signficant decreases in the incidence of seedling blights and angular-leaf-spot infections, compared to the checks, but acid delinting and flotation grading failed to give significantly better results than were obtained from the use of ethyl mercury phosphate dust on normal fuzzy seed.

INHERITANCE OF PATHOGENICITY IN MELAMPSORA LINI[1]

H. H. FLOR[2]

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INTRODUCTION

The flax-rust fungus, *Melampsora lini* (Pers.) Lév., is autoecious and long-cycle; that is, it produces its pycnial, aecial, uredial, and telial stages solely on species of flax. Allen (1) demonstrated the heterothallic nature of *M. lini*, and Flor (3, 4) found that rust of cultivated flax, *Linum usitatissimum* L., comprised numerous physiologic races.

Every flax variety tested has been susceptible to 1 or more of 24 physiologic races identified from North and South American collections of flax rust. However, no single race known is capable of attacking all of the 11 differential varieties used in these tests. In the northern United States, flax rust overwinters by means of telia (6) and, consequently, natural hybridization is probably coincident with the initiation of infection each year. A sound program for the production of desirable rust-resistant varieties of flax requires a knowledge not only of the interaction of the factors governing rust reaction possessed by the varieties of flax but also of the interaction of the factors for pathogenicity in various races of the flax-rust fungus. The data reported in the present paper deal with the latter subject.

Knowledge of the inheritance of pathogenicity in the rusts is limited. It was not until 1927 that Craigie (2) demonstrated the heterothallic nature of rusts and thus made possible the genetic study of these organisms. Physiologic races of rust are differentiated by the type of infection produced on a series of differential varieties, a laborious process restricting the number of progeny cultures that can be studied. Additional impediments to the genetic study of pathogenicity in the rusts are the capricious germination of the telia and the difficulty of obtaining races of known homozygosity. It also is probable that the differential varieties used to identify the physiologic races are not capable of resolving the rust into all of its genotypic strains.

New physiologic races of cereal rusts have been obtained by selfing or crossing known races of *Puccinia graminis tritici* Eriks. and Henn. (8, 12, 13, 16), *P. graminis avenae* Eriks. and Henn. (10), and *P. rubigo-vera tritici* (Eriks. and Henn.) Carl. (17), as well as by crossing the different varieties of stem rust (14). The data of Johnson, Newton, and Brown (8), obtained in a study of the inheritance of pathogenicity in a cross between two races (9a × 36) of *P. graminis tritici*, point toward Mendelian inheritance. In this cross the pathogenicity of F_2 rust cultures to Arnautka, Mindum, and Spelmar appeared to be inherited as a unit and was conditioned by a single pair of factors with virulence dominant. Pathogenicity to Kanred was conditioned by a single pair of factors with avirulence dominant, and pathogenicity to Vernal by 2 pairs of duplicate factors with avirulence dominant. Each of these 3 characters appeared to be inherited independently. In a cross between races of *P. graminis avenae,* Johnson and Newton (10) found that in the F_1 avirulence was dominant to virulence.

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[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the North Dakota Agricultural Experiment Station.

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MATERIAL AND METHODS

The races of *Melampsora lini* here reported were of diverse origin. Races 6, 9, and 10 originated from uredial collections made in Minnesota in 1934. Race 24 was obtained as a uredial collection from North Dakota in 1938. Race 20 was derived from a telial collection obtained from Uruguay in 1935, and race 22 from telia received from Argentina in 1937. The telia material used in these studies, with the exception of that of race 24 was directly descended from the single urediospore isolate from whose pathogenic properties the original race was described. The telia of race 24 were collected in the field in 1938 on Bombay, a variety that had been immune from all other races.

Considerable difficulty has been experienced in germinating teliospores produced in the greenhouse. Best success was obtained from telia developed on greenhouse-grown plants ripening in April, stored in the laboratory at room temperature for several weeks, frozen in blocks of ice for 6 months, and then alternately wetted and dried at 2-day intervals for several weeks. Even with this technique, germination has been exceedingly erratic, the teliospores of one race failing to germinate, although developed simultaneously and receiving treatment identical with those of a race that germinated profusely.

The capricious germination of the teliospores made it desirable to determine their viability prior to inoculating flax plants with sporidia. This was done by first wetting the segments of telia-bearing flax stems for 2 to 4 hours and then placing them on a glass slide in a Petri dish, with its cover lined with moist filter paper, and incubating overnight at 55–60° F. A microscopic examination of portions of the slide adjacent to the telia for the presence of sporidia revealed the state of activity of the teliospores.

In rust studies (1, 12) the essential feature of methods employed in inoculating plants with sporidia was to permit the gametophytic sporidia to shower down on a rust-susceptible plant in a moist atmosphere. In the studies here described satisfactory results were obtained by the following technique. The flax segments bearing the germinating teliospores were placed on a wet filter paper in the cover of a Petri dish which was inverted and stuck in the top of a large bell jar by means of a ball of moist clay. The bell jar, lined with moist filter paper, was placed over a 4-inch pot containing 5 vigorously growing rust-susceptible flax plants, 8 to 12 inches high. The plants were previously atomized with tap water until numerous fine water droplets formed on the leaves. The amount of infection was roughly controlled by observing the viability of the teliospores as shown by the preinoculation test and varying the quantity of telial material placed over the plants and the duration of the sporidial shower accordingly. As a rule, telial germination was poor and the plants were subjected to sporidial inoculation for 10 to 14 hours and then incubated in a moist chamber for an additional 12 hours.

Sporidial infection was first manifest as flecks on the leaves, usually 6 to 8 days after inoculation, the time varying with light and temperature conditions prevailing during the incubation period. Since cultures resulting from the transfer of pycnial nectar from one haploid infection to another comprised the basic unit in these studies, leaves having several pycnial infections were picked from the plant as soon as the pycnia were evident. Four to 6 additional days were allowed for the development of aecia from multiple infections not readily visible, before nectar was transferred from one pycnium to another. Except on heavily infected leaves, the development of normal aecia from what appeared to be single pycnial infections rarely occurred.

The amount of nectar produced by pycnia varied from almost microscopic droplets to drops so large that they dripped from the leaf. Usually, nectar production was relatively limited. The technique employed in transferring pycniospores from one haploid infection to another was as follows: A small loop of 28 gauge chromel wire was flamed, dipped in water or a solution of 5 per cent sucrose in water, and touched to a droplet of pycnial nectar. The nectar thus lifted was then transferred to a second pycnium. In attempted selfing, the usual procedure was to start at the base of the plant and transfer nectar to the lower pycnial infection from the just below the leaf bearing the infection receiving the nectar. By this pycnium immediately above it. A colored thread was tied around the stem method it was assured that pycniospores from a single pycnium would effect fertilization in not more than one aecium. This is essential in a genetic study of rusts as the paternal and maternal pustules contribute equally in Mendelian inheritance.

The aecia ruptured the epidermis of the leaf exposing the aeciospores in 2 to several days following transfer of nectar to a pycnium of opposite sex. Usually, in 5 days approximately 50 per cent of the matings produced vigorous aecia. As soon as the aecium was evident the leaf bearing it was plucked, laid on a glass slide, and the aeciospores dusted by means of a camel-hair brush on the unfolding leaves of the terminal bud of 3- to 4-inch seedlings of a rust-susceptible variety. The inoculated seedlings were atomized with tap water and incubated under bell jars for 18 to 24 hours at 55 to 60° F. The resultant urediospores were collected in glass vials and 4 to 8 seedlings of each of the rust-differentiating flax varieties were inoculated by dusting these spores on the leaves and terminal bud. The plants thus inoculated were placed in moist chambers, atomized with tap water, and incubated 24 hours. They were then removed to rust compartments in a greenhouse kept at about 70° F. during the day and 60° F. at night. To insure vigorous growth, daylight was supplemented during the hours of darkness with artificial illumination from 200-watt Mazda bulbs hung 12 inches above the plants.

The physiologic races of flax rust have been differentiated by the type of uredia produced on 11 varieties of flax (4). Infection type may be regarded as the visible expression of the reaction of the host to the parasite, a measure of either the susceptibility of the host or of the pathogenecity of the parasite. The presentation of the results has been facilitated by the use of the terms avirulent, semivirulent, moderately virulent, and virulent to describe types of rust infection, corresponding to classes of host reaction. Classes of host reaction and types of rust infection used in this paper are as follows:

Class of host reaction		Type of rust infection		
Immune	(I)	0	No uredia evident	Avirulent
Resistant	(R)	1, 2	Uredia small with chlorosis or necrosis	
Semi-resistant	(SR)	3–	Uredia variable with necrosis	Semi-virulent
Moderately susceptible	(S–)	1– to 3	Uredia small to medium; little necrosis, variable chlorosis, sensitive to environmental conditions	Moderately virulent
Susceptible	(S)	3 to 4	Uredia medium to large without chlorosis or necrosis	Virulent

Quantitative differences as shown by vigor of sporulation and number of pustules are indicated by plus (+) and minus (–) signs.

Resistant, semiresistant, and moderately susceptible reactions were considered to be "intermediate" in contrast with immune and susceptible.

Although hybridizing and selfing as applied to rusts may not be identical with the use of these terms as applied to spermatophytes, they are genotypically homologous. Instead of a single pollen-cell nucleus fusing with a single egg-cell nucleus, there probably occur numerous dikaryotic associations of the paternal and maternal nuclei. However, if the life history and nuclear behavior of *Melampsora lini* as reported by Allen (1) be true, all of the aeciospores, urediospores, and teliospores resulting from the transfer to one haploid pycnium of pycniospores from a second haploid pycnium of opposite sex are genotypically identical, except for possible mutations.

There are several sources of error in a genetic study of a rust such as *Melampsora lini*. It is not possible to ascertain that each pycnium is haploid and not the result of infection by two sporidia of the same sex. Also, the aecium of *M. lini* is caemoid, usually develops very rapidly, yet all aecial infections do not develop at the same rate. The aeciospores begin to shed as soon as the leaf epidermis covering the pustule ruptures and, consequently, there is the possibility of spores from one pustule falling on another. Attempts to catch the aecium just before the epidermis ruptured resulted in poor infection, loss of many cultures, and in obtaining numerous degenerate type cultures resembling the "subepidermal strains" of Johnson and Newton (9). Mixed cultures resulting from infections by two sporidia of the same sex but carrying different factors for pathogenicity, as well as those due to accidental contamination of one aecium with spores from another, could be detected if one of the differential varieties were resistant to one and susceptible to another of the races comprising the mixture. Detection was difficult if the races involved differed in pathogenicity on a variety that was either immune or susceptible (having no intermediate infection type), since only the susceptible type of pustule would be evident. Errors due to these sources would increase the proportion of virulent races, since a mixed culture would be read as having the pathogenicity of the more virulent race.

RESULTS

Selfed Parent Races

The reactions of the 11 differential varieties of flax, employed in identifying the physiologic races of the rust fungus, to the parental urediospore cultures and to the selfed cultures of each of the 6 races that have been studied are given in table 1.

The physiologic races of *Melampsora lini* were less heterozygous than were those of *Puccinia graminis tritici* and *P. graminis avenae,* as indicated by other studies (8, 10, 13). Three of the 6 races tested, namely, races 10, 22, and 24, appeared to be homozygous, as the reaction of the 11 rust differentiating flax varieties to all of the selfed cultures of each race agreed with their reactions to the parent race. Certain selfed cultures of each of races 6, 9, and 20, differed pathogenically from the parent races. Differences in pathogenicity of the progeny of these 3 pathogenically heterozygous races were in each case confined to 1 of the 11 differential varieties. However, a different variety was involved in each instance. Of the selfed cultures of race 6, 7 were like the parent to which Akmolinsk is resistant, and 2 differed from the parent only in producing a virulent type of infection on Akmolinsk. The range of pathogenicity of these 2 cultures was unlike that of any race previously described; therefore, a new number, namely, No. 33,[3] has been assigned to them. Of the 11 selfed cultures of race 9, 8 were like the parent race to which Williston Golden is moderately susceptible, and 3 were like race 13 to which this variety is resistant. Of the selfed cultures of race 20, 15 were like the parent race from which Buda is immune. Buda was susceptible to the other 4 cultures, which agreed in range of pathogenicity with race 19. In each case, the segregating ratio approximated

[3] Race numbers 25 to 32, inclusive, have been assigned to the 8 physiologic races identified by W. Straib, (Zum epidemischen Austreten des Leinrostes in Ostpreussen. Nachrichtenbl. f. den. Deut. Pflanzenschutzdienst 19: 49–51. 1939) from European collections of flax rust as follows: H–1, race 25; S–1, race 26; S–2, race 27; D–1, race 28; S–3, race 29; D–2, race 30; D–3, race 31; and D–4, race 32.

TABLE 1.—*Reaction of 11 differential varieties of flax to parent races of Melampspora lini and to the F_1 cultures of selfed races and hybrids*

Parent race, and selfed or hybrid F_1 culture	Number of cultures studied	Reaction of differential variety											Race of culture
		Buda C.I. 270–1	Williston Golden C.I. 25–1	Williston Brown C.I. 803–1	Akmolinsk C.I. 515–1	J. W. S. C.I. 708–1	"Pale blue crimped" C.I. 647	Kenya C.I. 709–1	Abyssinian C.I. 701	Argentine C.I. 462	Ottawa 770 B C.I. 355	Bombay C.I. 42	
Race 6		R[a]	S	S	R +	I	S –	R	I	I	I	I	6
Selfed race 6	2	R	S	S	S	I	[b]	R	I	I	I	I	33
Selfed race 6	7	R	S	S	R +	I	[b]	R	I	I	I	I	6
Race 9		S –	S –	S	R +	S	S –	R	I	I	I	I	9
Selfed race 9	3	S –	R	S	R +	S	R – to S –[b]	R	I	[c]	[c]	[c]	13[d]
Selfed race 9	8	S –	S –	S	R +	S	R – to S –[b]	R	I	[c]	[c]	[c]	9
Race 10		R+	R +	R	R +	I	S –	R +	I	I	I	I	10
Selfed race 10	11	R+	R +	R	R +	I	R[b]	R	I	[c]	[c]	[c]	10
Race 20		I	S	S	S	I	S	S	S	S	I	I	20
Selfed race 20	4	S	S	S	S	I	S	S	S	S	I	I	19
Selfed race 20	15	I	S	S	S	I	S	S	S	S	I	I	20
Race 22		S	S	S	S	I	S	S	S	S	S	I	22
Selfed race 22	9	S	S	S	S	I	S	S	S	S	S	I	22
Race 24		S –	S	S	R +	I	S –	R	I	I	I	S	24
Selfed race 24	15	S –	S	S	R +	I	S –	R	I	I	I	S	24
Race 9 × 10	13	SR to S –	R	S –	R +	I	R – to S –[b]	R –	I	[c]	[c]	[c]	17
10 × 9	19	SR	R	S –	R +	I	R[b]	R	I	[c]	[c]	[c]	17[d]
Race 6 × 22	1	SR	S	S	S	I	[b]	R	R –	I	I	I	34
6 × 22	2	SR	S	S	R +	I	[b]	R	R + to I	I	I	I	14
22 × 6	2	SR	S	S	S	I	[b]	R	R –	I	I	I	34
22 × 6	1	SR	S	S	R +	I	[b]	R	R + to I	I	I	I	14
6 × 24	2	SR	S	S	R +	I	[b]	R	I	I	I	I	14[d]
24 × 6	2	SR	S	S	R +	I	[b]	R	I	I	I	I	14[d]
22 × 24	1	S	S	S	R +	I	[b]	R	R + to I	I	I	I	2[d]
24 × 22	6	S	S	S	R +	I	[b]	R	R + to I	I	I	I	2[d]

[a] Plus and minus signs indicate somewhat greater or less resistance or susceptibility than the letter designating the host reaction. The letters signify the following: I, immune; R, resistant; SR, semi-resistant; and S, susceptible.

[b] These tests were conducted during the spring months and the reaction of "pale blue crimped" is abnormal.

[c] These differential varieties were not in use when tests were made.

[d] The reaction of several of the differential varieties to these F_1 cultures differed slightly from their reactions to the type race, but, because of the effect of environment on the intermediate reactions (R+, R, R–, SR, and S–), it has been considered inadvisable to differentiate races on the basis of differences in degree of reaction.

that that would have been expected were the parent race heterozygous for a single factor pair conditioning infection type on the variety to which the selfed cultures segregated for pathogenicity.

In order to determine the pathogenic range inherent in each selfed race, a mass transfer of pycniospores was made between the pycnia on heavily infected flax plants. The differential varieties were inoculated with a mixture of the resulting aeciospores and also with the urediospore increase resulting from the inoculation of a rust-susceptible variety with the aeciospore composite. In no instance did the composite aeciospore or urediospore collection have a wider range of pathogenicity than the selfed cultures of that race. The presence of races having a more restricted range of pathogenicity than that of the parent or the selfed cultures would not be apparent when a composite inoculum is used.

F_1 HYBRIDS BETWEEN RACES

Crosses between Races 9 and 10

Williston Golden was resistant to the 32 F_1 cultures of crosses between races 9 and 10. This is of special interest because selfing studies indicated that race 9 was heterozygous for pathogenicity to this variety, the factor for moderate virulence being dominant to that for avirulence. In the hybrid, apparently the factor of race 10 for avirulence to Williston Golden was dominant to the factor of race 9 for moderate virulence.

Repeated attempts to germinate telia, produced in the greenhouse, from crosses between races 9 × 10[4] and 10 × 9 have failed. However, significance cannot be attached to this, as parallel attempts to germinate some telia of races originating from urediospores collected in the field and subjected to identical growing conditions also failed.

Crosses between Races 6 and 22

Race 6 (Fig. 1, *A*) has a restricted pathogenicity. Williston Golden, Williston Brown, and "pale blue crimped" are the only differential varieties susceptible or moderately susceptible to it. Race 22 (Fig. 1, *B*) has the widest pathogenic range of all the races thus far identified. All of the differential varieties are susceptible to it except J.W.S. and Bombay, which are immune. None of the differential varieties has an intermediate reaction to race 22.

Selfing of race 6 showed it to be heterozygous for pathogenicity to Akmolinsk and indicated that avirulence was dominant and conditioned by a single pair of factors. Selfing of race 22 showed it to be homozygous for the production of the virulent infection type on Akmolinsk. The number of cultures of the cross between race 6 and 22 was limited by the paucity of pycnia of race 6. However, Akmolinsk was susceptible to 3 and resistant

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[4] Following the precedent of Newton, Johnson, and Brown (12), the race placed first (maternal) is the one on whose haploid pustules aecia are developed as the result of a transfer to them of pycniospore-containing nectar of the other (paternal) race.

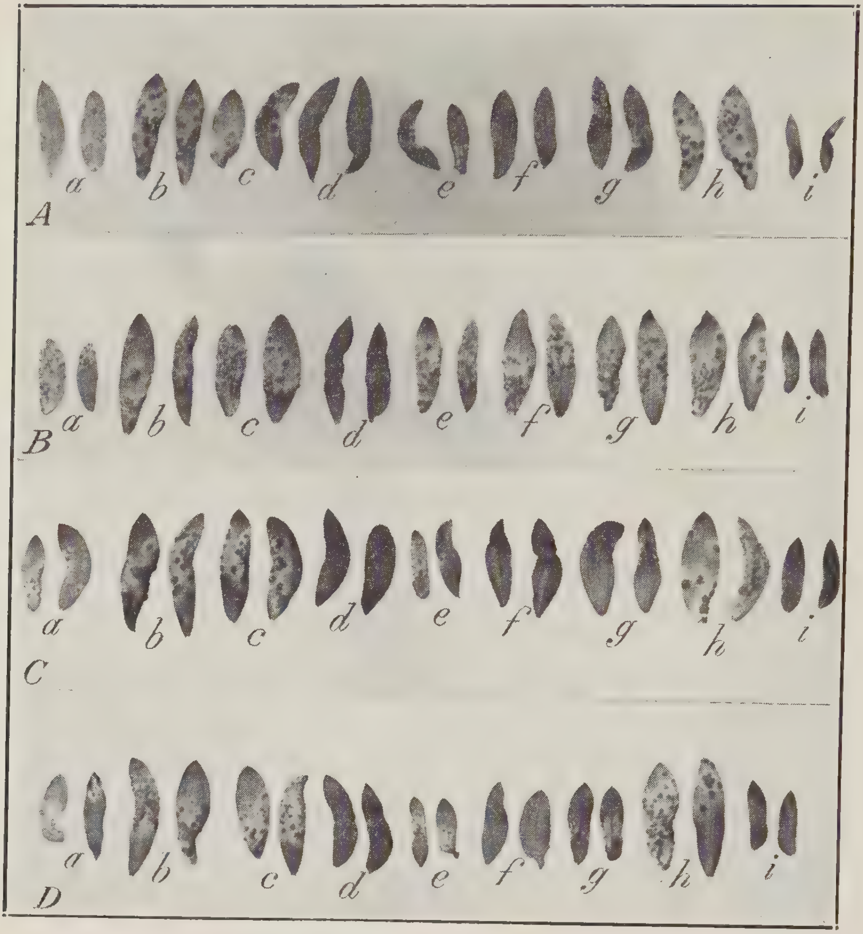

FIG. 1. Infection types produced by: *A*, race 6; *B*, race 22; *C*, F_1 of race 6 × race 22; *D*, F_1 of race 22 × race 6, on paired leaves of the flax varieties: *a*, Buda; *b*, Williston Golden; *c*, Akmolinsk; *d*, J.W.S.; *e*, Abyssinian; *f*, Ottawa 770B; *g*, Argentine; *h*, Bison, and *i*, Bombay.

to 3 of the 6 F_1 hybrid cultures. This is the ratio that would be expected if race 6 were heterozygous and race 22 homozygous for a pair of factors conditioning ability to produce a virulent infection type on Akmolinsk.

Buda was semiresistant to the 6 F_1 cultures of hybrids between races 6 and 22, a reaction intermediate to that of this variety to the parent races. The F_1 cultures of this cross were identical in pathogenicity except for the infection types on Akmolinsk and Abyssinian. Three of the 6 F_1 cultures had a range of pathogenicity resembling that of race 14 and similar to that of the F_1 hybrid cultures of race 24 × race 6 (Fig. 2, *C*). Akmolinsk was resistant and Abyssinian was highly resistant to or immune from these cultures. The other 3 cultures (Fig. 1, *C* and *D*), to which race number 34 has been assigned, had a range of pathogenicity different from that of any previously described race. To these, Akmolinsk was susceptible and Abyssinian moderately resistant.

In all tests thus far made, Abyssinian has been highly resistant to or immune from all races to which Akmolinsk has been resistant; and moderately resistant, moderately susceptible, or susceptible to races to which Akmolinsk has been susceptible. Akmolinsk has been susceptible to all races attacking Abyssinian but the latter has been resistant to certain races to which the former has been susceptible.

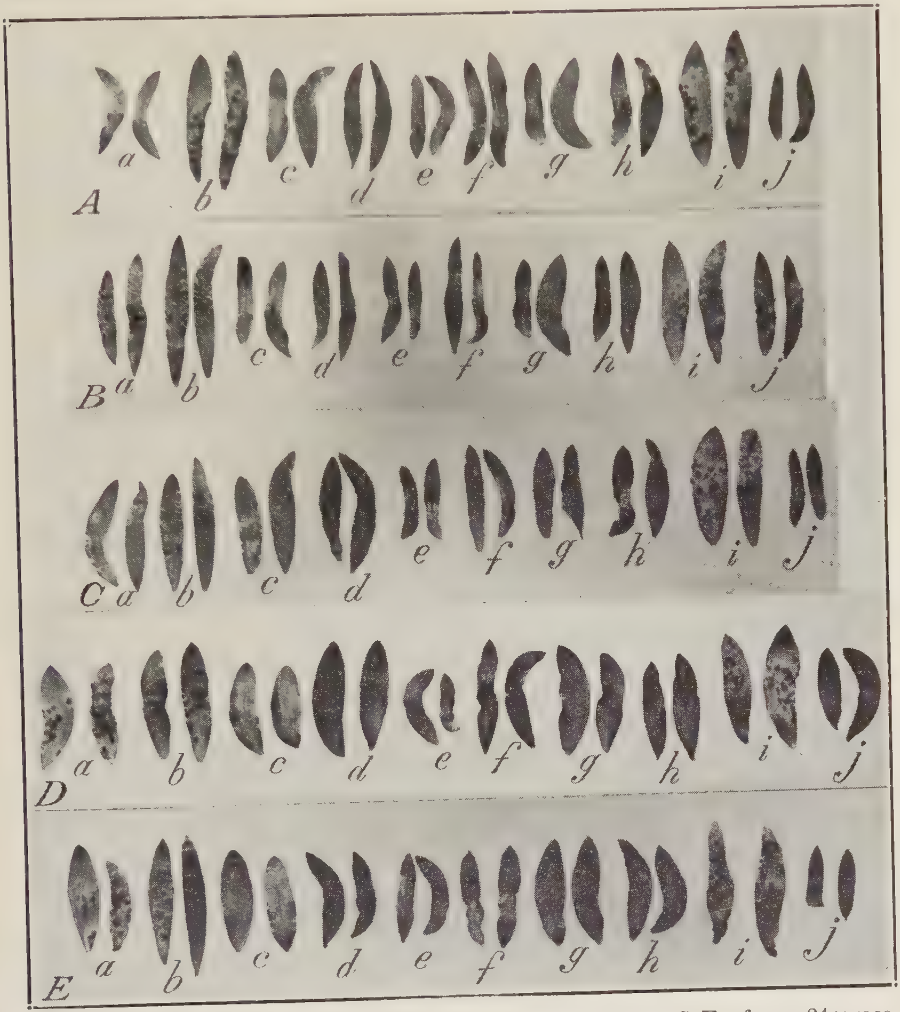

FIG. 2. Infection types produced by: *A*, race 6; *B*, race 24; *C*, F_1 of race 24 × race 6 (Race 14); *D*, F_1 of race 22 × race 24; and *E*, F_1 of race 24 × race 22, on paired leaves of the flax varieties: *a*, Buda; *b*, Williston Golden; *c*, Akmolinsk; *d*, J.W.S.; *e*, Abysinnian; *f*, Kenya; *g*, Ottawa 770B; *h*, Argentine; *i*, Bison, and *j*, Bombay.

Crosses between Races 22 and 24

Races 22 and 24 differ distinctly in pathogenicity to several of the differential varieties. All the differential varieties are susceptible to race 22 except J.W.S. and Bombay, which are immune (Fig. 1, *B*). Bombay is susceptible, and Akmolinsk, Kenya, Abyssinian, Argentine, and Ottawa 770B are resistant to or immune from race 24 (Fig. 2, *B*).

The range of pathogenicity of the F_1 cultures conformed to the pattern of the other hybrids. Williston Golden and Williston Brown, susceptible to both parent races, were susceptible to the F_1 hybrids. Buda, moderately susceptible to race 24 and susceptible to race 22, was more susceptible to the F_1 hybrids than to the race 24 parent (Fig. 2, *D, E*). Akmolinsk and Abyssinian were as resistant to the F_1 hybrids as they were to race 24. J.W.S., immune from both races, also was immune from the F_1 hybrids. Argentine and Ottawa 770B, immune from race 24 but susceptible to race 22, and Bombay, immune from race 22 but susceptible to race 24, were immune from the F_1 hybrids. The F_1 hybrids had a range of pathogenicity resembling that of race 2. Thus, in these crosses, as in the preceding ones, the factors for avirulence appeared to be dominant.

Crosses between Races 6 and 24

The crosses between races 6 and 24 were made in the spring of 1939 and only 4 F_1 cultures survived over-summer storage. The parents, race 6 (Fig. 2, *A*) and race 24 (Fig. 2, *B*), differ only in pathogenicity to Buda and Bombay. These 2 varieties are, respectively, highly resistant to and immune from race 6; and moderately susceptible and susceptible, respectively, to race 24. The hybrids had the pathogenicity of race 14. As in the crosses already discussed, the reaction of Buda to the F_1 hybrid cultures was somewhat intermediate to its reaction to the parent races. The reaction of Buda to these hybrids, although approaching its reaction to race 24, was distinctly less susceptible (Fig. 2, *C*).

F_2 Cultures of Race 6 × Race 24

One of the F_1 cultures of race 6 × race 24 was selfed and the distribution of the pathogenic characters in which the parent races differed was determined for 96 F_2 cultures. The F_1 cultures segregated for pathogenicity to Akmolinsk, as well as to Buda and Bombay. As previously mentioned, although Akmolinsk was resistant to both parent races, it was susceptible to some of the selfed cultures of race 6; an indication that the parent race 6 was heterozygous for pathogenicity to this variety. Apparently, the haploid pycnium of race 6 fertilized by pycniospores of race 24, from which this hybrid culture developed, possessed the factor for virulence to Akmolinsk. The reaction of the other 8 differential varieties to the F_2 cultures of this cross was identical with their reaction to the parental and F_1 cultures.

The reaction of Akmolinsk and Bombay to all cultures of *Melampsora lini* has been clear cut. The former has been either highly resistant or susceptible and the latter either immune from or susceptible to all races with which they have been tested. The reactions of Buda to the several races are diverse, varying by almost imperceptible stages from immunity to susceptibility. Since the reaction of Buda is influenced by environment, tests with this variety were repeated. To assure uniform environmental conditions during the period of rust development, the reaction of Buda to the 96 F_2 cultures was determined simultaneously. After inoculation and incubation, the pots were placed on a greenhouse bench and rotated daily so as to subject each pot of inoculated plants to relatively uniform light conditions. When the infection types had fully developed, the entire series was arranged according to severity of infection. A natural division seemed to be into the following 5 classes, based on type and degree of infection: (1) resistant (R); (2) moderately resistant (R–), intermediate between the resistant and semiresistant classifications having pustules more numerous and vigorous than the former and smaller than those of the latter; (3) semiresistant (SR); (4) slightly susceptible (SR–) intermediate between the semiresistant and the moderately susceptible classes of host reaction, having more numerous, medium-size pustules of greater vigor than the semiresistant class but accompanied by distinct necrotic lesions; and (5) moderately susceptible (S–).

The frequency distribution of the reaction of Buda, Akmolinsk, and Bombay to the F_2 progeny of the cross between race 6 and race 24 is given in table 2. Apparently, 2 pairs of factors are involved in conditioning pathogenicity to Buda. On this basis it is assumed that avirulence is obtained in the segregates in which the two dominant factors are homozygous and moderate virulence when the 2 recessive factors are homozygous and intermediate types when either or both pairs of factors are heterozygous. Thus the ob-

TABLE 2.—*Segregation in the F_2 of a cross between race 6 and race 24, for pathogenicity to the varieties Buda, Akmolinsk, and Bombay*

Variety	Class of host reaction	Infection type	Number of F_2 cultures producing indicated infection type			
			Calculated		Observed	
					a	b
Buda	Resistant	1		6		5
Buda	Intermediate:					
	Moderately resistant	1 to 2+	24	84	24	84
	Semiresistant	1, 2 and 3–	36		42	
	Slightly susceptible	1, 2+ and 3–	24		18	
	Moderately susceptible	1+ to 3		6		7
Akmolinsk	Resistant	1	72			[c] 71
Akmolinsk	Susceptible	3 to 4	24			25
Bombay	Immune	0	72			[d] 67
Bombay	Susceptible	3 to 4	24			29

[a] X^2 for a ratio of infection types on Buda of 1 *type 1*: 4 *types 1 to 2+*: 6 *types 1, 2 and 3–*: 4 *types 1, 2+ and 3–*: 1 *types 1+ to 3* = 2.83. *P* lies between 0.50 and 0.70.

[b] X^2 for a ratio of infection types on Buda of 1 *type 1*: 14 *types 1 to 3–* (intermediate): 1 *types 1+ to 3* = 0.33. *P* lies between 0.80 and 0.90.

[c] X^2 for a ratio of infection types on Akmolinsk of 3 *type 1*: 1 *types 3 to 4* = 0.056. *P* lies between 0.80 and 0.90.

[d] X^2 for a ratio of infection types on Bombay of 3 *type 0*: 1 *types 3 to 4* = 1.39. *P* lies between 0.20 and 0.30.

served segregating ratio of the 96 F_2 cultures for pathogenicity to Buda closely approaches the theoretical 1:14:1, *P* lying between 0.80 and 0.90. Although division into the intermediate reaction classes was arbitrary, the observed ratio of segregates approached that which would be expected if ability to cause Buda to have (1) a moderately resistant reaction (R–) was conditioned by 3 dominant and 1 recessive factors, (2) a semiresistant reaction (SR) by 2 dominant and 2 recessive factors, and (3) a slightly susceptible reaction (SR–) by 1 dominant and 3 recessive factors, *P* lying between 0.50 and 0.70.

The segregating ratios obtained for pathogenicity to Akmolinsk and Bombay satisfactorily fit expectation if the virulent infection type is conditioned, in each instance, by an independent pair of recessive factors, *P* for Akmolinsk lying between 0.90 and 0.95, and *P* for Bombay lying between 0.20 and 0.30.

The factors conditioning virulence to Akmolinsk appeared to be linked with one of the pairs of factors conditioning avirulence to Buda. To the 25 F_2 cultures to which Akmolinsk was susceptible, Buda was either resistant, moderately resistant, or semiresistant. In table 3 are given the theoretical distribution of genotypes and the observed segregation for classes of reaction, assuming that single independent pairs of recessive factors condition ability to produce the virulent type of pustule on Akmolinsk and Bombay and that two pairs of quantitative factors condition the infection type on Buda with very close or complete linkage between the factors conditioning virulence to Akmolinsk and one of the pairs of factors conditioning avirulence to Buda.

The infection types of 1 of the 96 F_2 cultures did not conform to the assumed complete linkage between a resistant infection type on Buda and a susceptible infection type on Akmolinsk. Theoretically, both Buda and Akmolinsk should not be resistant to any of the F_2 cultures. Actually, one such culture did occur. This may be accounted for by crossing over, by mutation, or by failure to express normal infection type because of environment. Since Buda was distinctly resistant to this culture in both tests, the crossing over or the mutation theories seem more plausible. Ignoring this one culture, the *P* value for observed segregation for pathogenicity according to theoretical genotypes was about 0.50, indicating good agreement of the data with the hypothesis.

The occurrence of intermediate classes of reaction on Buda, together with its sensitiveness to changes in environment makes this variety somewhat unsatisfactory as a rust differential. The following classes of reaction of Buda have served to differentiate physiologic races of the flax-rust organism: (1) Resistant (R), including moderately resistant (R–) and highly resistant (R+); (2) semiresistant (SR); and (3) susceptible (S), including moderately (S–) and highly susceptible (S+). When F_2 cultures to which Buda is slightly susceptible (SR–) are grouped with those to which it is semiresistant, the actual distribution of physiologic races comprising the 96 F_2 cultures closely approaches the theoretical, *P* lying between 0.50 and 0.70.

TABLE 3.—*Genotypes and segregation for pathogenicity to the flax varieties Buda, Akmolinsk, and Bombay, and for physiologic races in the* F_2 *of a cross between races 6 and 24*

Assumed genotypes[a]	Reaction to F_2 culture of			Number of cultures having indicated genotype based on pathogenicity			Physiologic race number	Distribution of physiologic races	
	Buda	Akmolinsk	Bombay	Calculated		Observed[b]		Calculated	Observed[c]
F_2 Genotype:									
AABC/Bc(C)Dd(D)	R	R	I		0.0	1	6	9.0	9
AABc/bCDd	R–	R	I	6.0	9.0	8			
AABc/bCDD				3.0					
AAbC/bCDd	SR	R	I	3.0	22.5	26	14	40.5	37
AAbC/bCDD				1.5					
AaBc/bCDd				12.0					
AaBc/bCDD				6.0					
AabC/bCDd	SR–	R	I	6.0	18.0	11			
AabC/bCDD				3.0					
aaBc/bCDd				6.0					
aaBc/bCDD				3.0					
aabC/bCDd	S–	R	I	3.0	4.5	3	2	4.5	3
aabC/bCDD				1.5					
AABc/BcDd	R	S	I	3.0	4.5	2	33	13.5	13
AABc/BcDD				1.5					
AaBc/BcDd	R–	S	I	6.0	9.0	11			
AaBc/BcDD				3.0					
aaBc/BcDd	SR	S	I	3.0	4.5	5	34	4.5	5
aaBc/BcDD				1.5					
AABc/bCdd	R–	R	S		3.0	3	35	3.0	3
AaBc/bCdd	SR	R	S	6.0	7.5	8	36	13.5	15
AAbC/bCdd				1.5					
AabC/bCdd	SR–	R	S	3.0	6.0	7			
aaBc/bCdd				3.0					
aabC/bCdd	S–	R	S		1.5	4	24	1.5	4
AABc/Bcdd	R	S	S		1.5	2	37	4.5	4
AaBc/Bcdd	R–	S	S		3.0	2			
aaBc/Bcdd	SR	S	S		1.5	3	38	1.5	3

[a] Assumed genotype: parent gamete of race 6, ABcD; of race 24, abCd; and of F_1 culture, AaBbCcDd.
[b] $\chi^2 = 12.02$, *P* lies between 0.50 and 0.70. [c] $\chi^2 = 6.767$, *P* lies between 0.50 and 0.70.

The data in table 3 can be explained on a factorial basis by assuming that the haploid pustule of race 6 contributed the quantitative factors AB conditioning avirulence to Buda, the recessive factor c conditioning virulence to Akmolinsk, and the dominant factor D conditioning avirulence to Bombay, and the pycniospores from the haploid pustule of race 24 contributed the quantitative factors ab, conditioning moderate virulence to Buda, the dominant factor C conditioning avirulence to Akmolinsk, and the recessive factor d conditioning virulence to Bombay, with linkage between the factors Bc and bC. The 10 physiologic races theoretically possible on the basis of this assumption were obtained, of which 6, namely races 33, 34, 35, 36, 37, and 38, had not been isolated prior to these studies.

CYTOPLASMIC INHERITANCE

Some evidence of cytoplasmic inheritance of certain pathogenic characters has been reported in crosses between certain races of *Puccinia graminis tritici* (13) and *P. graminis avenae* (10). In those cases certain pathogenic characters of the maternal parent, which could not be accounted for on a strictly Mendelian basis, were evident in the F_1 and subsequent generations. In the present studies, in addition to making crosses in both directions, pycnial nectar was exchanged between single haploid pustules of the races hybridized. Only in the cross between races 9 and 10 was there any indication of cytoplasmic inheritance. Buda appeared to be slightly more susceptible to F_1 cultures of 9×10 than to those of 10×9.

DISCUSSION

The development of rust-resistant varieties of flax is complicated by the existence of numerous physiologic races of the flax-rust fungus. The physiologic races occurring in different regions differ in range of pathogenicity. For instance, Punjab was susceptible in Minnesota and North Dakota in 1940 but has been immune from rust in Argentina, while Ottawa 770B is susceptible in Argentina (15) but has been immune in North America (4). The physiologic races prevalent in a given locality may differ from season to season. In 1938 and 1940 Bombay was susceptible to rust at Fargo, N. Dak., but in 1939 this variety was immune from the physiologic races occurring there. Some of the fundamental problems in the study of a plant disease, such as flax rust, are the determination of (1) the range of pathogenicity of the causal organism, (2) if and how new strains come into existence, (3) the potential range of pathogenicity of these new strains, and (4) the interaction between factors for pathogenicity in the pathogen and those for resistance in the host. These problems involve genetic studies of the pathogen as well as of the host varieties that exhibit a differential response to the physiologic races of the pathogen. The determination of the pathogenic capacities of the selfed and hybrid cultures that have been presented in this paper is a preliminary step in such a study.

The determination of the interaction of factors for pathogenicity in the flax-rust fungus and those for resistance in the host is complicated by the occurrence of numerous pathogenically distinct physiologic races of the former as well as by the existence of numerous strains of the latter possessing distinct factors for resistance. It is known that a single factor in J.W.S. conditions immunity from at least 18 physiologic races of *Melampsora lini* and that other flax varieties possess several independent factors for immunity from or resistance to specific physiologic races (5, 11). At least several of the factors for immunity and resistance appear to occur in allelomorphic series. Consequently, it has not been possible to obtain a strain of flax homozygous for certain combinations of the resistant and immune factors. It would be of value to know if parallel conditions exist in the pathogen. Is the ability of a virulent race to attack a number of flax varieties, known to possess different factors for rust reaction, due to one or to a number of factors each of which overcomes a specific "resistance" factor in the host? If numerous pathogenic factors are involved may not some of them be allelomorphic?

The data in the present paper indicate that the range of pathogenicity of a physiologic race of *Melanspora lini* is determined by pathogenic factors specific for each resistance factor possessed by the host. Henry (7) reported that in Bombay immunity from North American collections of flax rust was conditioned by a single pair of factors. In the present studies, pathogenicity to Bombay was conditioned by a single pair of factors with avirulence dominant. Unpublished data of the writer show that the reaction of Akmolinsk to the physiologic races used in these studies is conditioned by a single pair of factors. Pathogenicity to Akmolinsk of the selfed and hybrid cultures also was conditioned by a single pair of factors, avirulence being dominant, independent of the factors for pathogenicity to Bombay.

Flor (5) found that 2 pairs of factors were involved in conditioning reaction of Buda to rust. Both pairs were operative as incomplete dominants or quantitative factors to races to which Buda was resistant, while only one of the factor pairs was operative to race 20, from which Buda was immune. Selfing studies of race 20 indicated that it was heterozygous for pathogenicity to Buda and that the virulent type of infection was recessive and conditioned by a single pair of factors. To the F_1 hybrids between races to which it was susceptible and those to which it was resistant, Buda produced an intermediate infection type indicative of a lack of dominance of the factors for pathogenicity. The segregating ratio of the F_2 cultures of such a hybrid indicated that 2 pairs of quantitative factors conditioned pathogenicity to Buda.

SUMMARY

The inheritance of pathogenicity in *Melampsora lini* was studied by selfing and hybridizing physiologic races.

Three of the six physiologic races selfed, namely races 10, 22, and 24, were apparently homozygous for pathogenicity, the infection types of each

selfed culture being identical with those of its parent on the 11 differential flax varieties. The three other races, 6, 9, and 20, were heterozygous, some selfed cultures of each race differing from the parent in pathogenicity when tested on the differential varieties.

Avirulence was invariably dominant in crosses between physiologic races, although, on Buda, dominance was incomplete. Consequently, the F_1 hybrid resembled the weaker of the two parents in pathogenicity or combined the less virulent characteristics of each. The range of pathogenicity of a uredial culture does not necessarily indicate its genotype. The F_1 uredial cultures of a cross between the virulent races 22 and 24 had the pathogenicity of race 2, a race of relatively restricted pathogenic range.

The F_2 cultures of a cross between race 6 and race 24 segregated for infection types on Buda, Akmolinsk, and Bombay. The segregation for pathogenicity of these cultures was explained by assuming that, in this hybrid, 2 pairs of quantitative factors conditioned infection type on Buda, that single independent pairs of factors, with avirulence dominant, conditioned infection types on Akmolinsk and Bombay, and that the pair of factors conditioning infection type on Akmolinsk was linked with one of the pairs of factors for pathogenicity to Buda. The 10 physiologic races theoretically possible from the assumption were isolated. Six of these had not been described previously.

The pathogenic characters of physiologic races obtained by selfing and hybridizing could be accounted for by Mendelian segregation and recombination of pathogenic characters inherent in the parent races.

Pathogenicity of the selfed and hybrid cultures to Akmolinsk and Bombay, varieties in which single pairs of factors determine reaction to rust, was conditioned by single pairs of independent factors. Pathogenicity to Buda, a variety possessing 2 pairs of factors for resistance to race 6, was conditioned by 2 pairs of factors in the hybrid between race 6 and race 24. Only 1 of the 2 pairs of factors in Buda is effective in conditioning rust reaction to race 20, which was heterozygous for pathogenicity to this variety. The selfed cultures of race 20 segregated for pathogenicity in a single factorial ratio when tested on Buda. These facts suggest that the pathogenic range of each physiologic race of the pathogen is conditioned by pairs of factors that are specific for each different resistant or immune factor possessed by the host variety.

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STUDIES ON THE PATHOGENICITY OF FUSARIUM SPECIES ASSOCIATED WITH ROOT ROT OF WHEAT[1]

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INTRODUCTION

A root disease of wheat and other cereals caused by *Helminthosporium sativum* P. K. and B. and *Fusarium* spp., and usually referred to as common root rot, is very prevalent and destructive in the three prairie provinces of Canada. Recently, Simmonds (31) gave a good description of the symptoms of this disease, and called attention to its economic importance in Western Canada.

Although *Helminthosporium sativum* is by far the most important fungus associated with common root rot of small grain crops, particularly of wheat and barley, *Fusarium culmorum* (W. G. Sm.) Sacc., and several other species of *Fusarium*, have been consistently isolated from root-rotted specimens of cereals and grasses. For instance, Simmonds (28), Greaney and Bailey (14), and Broadfoot (7) report that, in Western Canada, the crowns and basal parts of wheat plants are frequently infected with *Fusarium* spp. Bolley (6) and Atanasoff (1) claim that many different species of *Fusarium* cause root rot of wheat and other cereals in the United States. Bennett (3, 4) isolated and identified 14 different species of *Fusarium* from British cereals. Russell (22), in 1930, and Samuel and Greaney (24), in 1935, obtained several species of *Fusarium* from the roots and stem bases of wheat plants collected from different fields in England. Shen (27) reported that brown foot rot of cereals, caused by *Fusarium* spp., was unusually prevalent in certain districts of England in 1938. According to Gordon and Sprague (12) one of the most common genera of fungi associated with gramineous root rots in the Northern Great Plains of the United States is *Fusarium*.

From the studies of Waksman (32), Beckwith (2), Henry (17), Reinking and Manns (20, 21), Sadasivan (23), and others, it would appear that fungi belonging to the genus *Fusarium* are regularly occurring soil inhabitants. Recent studies on the microflora of the soil carried out at this Laboratory have shown that these fungi are extremely common in grain soils. According to Gordon (11), species of *Fusarium* accounted for 10.8 per cent of 13,800 isolations of fungi obtained from the soil of permanent grain plots at Winnipeg in 1936. In 1937, they comprised 18.2 per cent of the total isolations. Many species, varieties, and forms, belonging to 8 sections of the genus *Fusarium*, were represented.

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Plant disease surveys and field experiments carried out for a number of years in Western Canada have indicated that the incidence of Helminthosporium-Fusarium root rot of wheat varies greatly from year to year. The cause of this variation has been attributed to such factors as the occurrence of physiologic forms of the pathogens, differences in soil and climatic conditions, and the association effects of other soil-inhabiting microorganisms. Garrett (9) has given an adequate review of the literature on factors affecting the pathogenicity of cereal root- and foot-rot fungi, and the results of earlier studies on this subject in Canada have been summarized by Simmonds (29, 30).

Many different species of *Fusarium* usually are associated with seedling blight and root rot of wheat. Obviously, therefore, a knowledge of the parasitic capabilities of these different fungi is the essential basis for the development of permanent control measures. Furthermore, for the effective control of Fusarium root rot of wheat and other cereals it is important to have a thorough knowledge of the conditions that favor the development of parasitic species of *Fusarium* in the soil, and influence the susceptibility of their host plants.

The objects of the present experiments were (i) to determine the relative virulence of different cereal isolates of *Fusarium*, particularly *F. culmorum*, on wheat, and (ii), to study the influence of soil moisture, soil temperature, and of certain common soil-inhabiting fungi on the pathogenicity towards wheat in the seedling stage of the 5 species of *Fusarium* most frequently isolated from the roots and crowns of cereal plants and from grain soils in Manitoba.

MATERIALS AND METHODS

A list of the species of *Fusarium* and isolates of *F. culmorum* used in the investigation is given in table 1, which contains also the origin of the fungi and the date of their isolation.

Greenhouse pot tests were used to study the pathogenicity of the abovementioned fungi to wheat seedlings. Inoculum of each organism was prepared by growing the fungus in flasks on a mixture of sterilized sand and cornmeal (2 per cent) for about 15 days. This sand-cornmeal inoculum was then mixed with autoclaved soil, 1 part of inoculum to 30 parts of soil, by weight, and the soil mixture placed in four 6-inch flower pots. Before planting, the soil in each pot was brought to a uniform moisture content of 50 per cent of its total water-holding capacity and weighed. This moisture content was maintained throughout the period of the experiment by weighing the pots at 2-day intervals and adding enough sterile water to bring them back to their original weight. In order to allow the fungus to become well established in the soil, 3 days were allowed to elapse between the time of soil inoculation and date of planting.

Twenty-five seeds of Marquis wheat were planted per pot. Before planting, the seed was surface-sterilized and washed in sterile water. It was then inoculated with a water suspension of spores of the fungus to be tested. The control seed for each experiment was dipped in sterile water. After planting, the pots of each experiment were randomized on a large bench in the centre of the greenhouse.

In order to investigate the effect of soil moisture, soil temperature, and other soil-inhabiting fungi on the pathogenicity of *Fusarium* species to wheat seedlings, Marquis wheat was inoculated with *F. culmorum* (W. G. Sm.) Sacc., *F. redolens* Wr., *F. avenaceum* (Fr.) Sacc., *F. oxysporum* Schl. v. *aurantiacum* (Lk.) Wr., and *F. equiseti* (Cda.) Sacc., respectively, in 6-inch pots of soil. The methods used to inoculate the seed and soil were essentially similar to those described above. Additional methods, introduced to meet the requirements of these specific experiments, will be described in later sections of this paper.

TABLE 1.—*Source of species and isolates of Fusarium*

Organism	Isolate No.	Year isolated	Host	Locality
Fusarium redolens	26	1935	Wheat	Morden, Man.
" *oxysporum*	73	1937	"	Winnipeg, Man.
" *avenaceum*	66	1936	"	" "
" *equiseti*	94	1937	"	" "
Fusarium culmorum	19A	1930	"	Saskatoon, Sask.
	19B	"	"	" "
	19C	"	Oats	" "
	38	1932	Wheat	Rhodes, Man.
	39	"	"	" "
	529	1933	Brome Grass	Winnipeg, Man.
	854	1935	Barley	Emerson, Man.
	856	"	Wheat	Bowsman, Man.
	937	"	"	Pilot Mound, Man.
	1040	"	"	Brandon, Man.
	1111	"	"	Clearwater, Man.
	1119	"	Oats	Melita, Man.
	1122	"	Wheat	Deloraine, Man.
	1158	"	"	Binscarth, Man.
	1193	"	"	Foxwarren, Man.
	1197	"	"	Virden, Man.
	1202	"	"	Shoal Lake, Man.
	1263	"	"	Bowsman, Man.
	1280	"	"	Griswold, Man.
	1300	"	"	Pipestone, Man.
	6	1936	"	Pope, Man.
	10	"	"	Harpenden, Eng.
	15	"	"	New Norway, Alta.
	285	"	"	Saskatoon, Sask.

In all greenhouse experiments, germination was usually complete 12 days after planting, at which time the number of emerged plants per pot series was counted. The seedlings were harvested and the degree of infection on each plant was recorded 4 to 5 weeks after planting. The infection classes and the disease rating formula used in these studies were the same as those employed by Greaney (15) in previous pot tests with *Fusarium culmorum*.

After the disease data had been recorded, the total green weight per pot series was taken.

In addition to seedling tests, studies were made to determine the relative pathogenicity of the above-mentioned species of *Fusarium* to adult wheat plants. The methods used for this purpose were briefly as follows. Surface-sterilized seeds of Marquis wheat were sown in sterilized quartz sand. When the seedlings were 6 days old they were transplanted from the sterilized sand to 6-inch pots of soil containing inoculum of the fungus to be tested. At the time of transplanting the entire root system of the young seedlings was inoculated by dipping it in a water suspension of spores and mycelial fragments of the respective fungi. Five inoculated seedlings were then placed in each of 5 pots of inoculated soil. Thereafter, the soil of each fungus series was inoculated with a spore suspension at regular intervals (10, 20, 50, and 70 days) during the growing period. The moisture and temperature of the soil were maintained at fairly constant levels in all pots throughout the experiment. The plants were harvested at maturity and the weight of plants and yield of grain recorded. The intensity of disease on each plant, and on the plants of each fungus series, was recorded according to the methods described by Greaney *et al.* (16). The experiment was run at 2 different times.

A field experiment was conducted in 1939 to determine the relative virulence on mature wheat plants of *Fusarium redolens, F. avenaceum, F. oxysporum* and *F. equiseti,* and 9 selected isolates of *F. culmorum.* Surface-sterilized seed of Renown wheat was inoculated with spores of the fungus to be tested, and planted in plots of artificially infested soil. The complete experiment consisted of 4 replicates of 16 plots each. Data on emergence were taken 23 days after planting, and notes on the incidence of disease were taken just before the plants ripened. One rod row of each plot was harvested for yield data. The methods used to infect the soil and record the amount of root-rot infection on individual plants were those employed in earlier field studies with cereal root-rot fungi (16).

The plant emergence, disease, and yield data of each greenhouse and field experiment were treated statistically by the analysis of variance method, and the significance of the observed differences between organisms and soil treatments determined.

EXPERIMENTAL RESULTS

Pathogenicity of Species and Isolates of Fusarium

Greenhouse Experiments. The results of greenhouse experiments designed to determine the relative pathogenicity to wheat seedlings of 24 different isolates of *Fusarium culmorum* are given in table 2. The data of both seedling and adult plant pathogenicity tests with 5 different species of *Fusarium* are summarized in table 3. To economize space, the complete analyses of variance data for these greenhouse experiments are not given. For each experiment, however, the results of the analyses showed highly significant differences between organisms for all of the properties measured.

It is evident from table 2 that a wide variation exists in the ability of isolates of *Fusarium culmorum* to attack young wheat plants. In view of the great susceptibility of wheat seedlings growing in sterilized re-infested soil to root-rot fungi, the present test must be considered a severe one. Nevertheless, even under the most favorable conditions for infection, the great majority of the 24 isolates tested were either non-pathogenic or only weakly pathogenic to Marquis wheat. One isolate of *F. culmorum,* No. 38, exhibited extreme virulence, while a few others, including Nos. 937, 1263, and 19C, were moderately pathogenic. The present results are in agreement with those of Sanford and Broadfoot (26), and show that the great majority of the isolates of *F. culmorum* obtained from cereal plants and from grain soils are weakly, if at all, pathogenic to young wheat plants.

TABLE 2.—*The relative pathogenicity to Marquis wheat seedlings of different isolates of Fusarium culmorum*

(Data are means of 4 trials)

Isolate of *Fusarium culmorum*[a]	Percentage of plants emerged	Disease rating	Green weight of plants (grams)
38	47.0	64.0	42.6
937	59.8	35.9	60.9
1263	57.2	35.8	63.2
19C	68.5	34.2	62.5
1300	63.0	32.2	68.5
1202	66.0	28.3	61.0
10	61.8	27.8	68.5
1040	59.2	25.8	62.4
1119	63.0	25.1	67.3
854	61.2	24.6	66.0
1280	63.0	24.4	70.0
6	64.0	23.8	69.0
15	67.5	22.9	74.6
1158	66.2	22.4	71.6
1193	65.0	21.1	66.7
856	60.2	20.6	67.6
1111	67.2	19.5	71.2
1197	78.5	17.6	84.0
19B	64.5	16.8	73.3
19A	75.2	16.4	78.0
1122	72.2	16.0	76.7
39	74.0	11.9	78.3
285	77.0	10.0	79.0
529	70.0	6.0	80.3
Control A	88.7	2.8	91.2
Control B	90.6	3.0	92.1
Necessary difference, 5% level	10.8	10.1	13.3

[a] Isolates arranged in order of virulence.

In the final analysis of this experiment, disease ratings and total green weights for the plants of the individual fungus series were correlated. The significance of the coefficient obtained was determined by the method described by Goulden (13). A highly significant correlation coefficient of -0.9259 was obtained. This result indicated that root-rot infection was very closely associated with plant growth, the higher the degree of infection the poorer was the development of the young wheat plants.

It is evident from table 3 that, of the 5 species of *Fusarium* tested, only *F. culmorum* (Isolate No. 38) was markedly pathogenic to Marquis wheat in both the seedling and post-seedling stages of plant growth. *F. avenaceum* and *F. equiseti* were nonpathogenic, or only very weakly pathogenic, to wheat seedlings, but attacked plants in the post-seedling stage of growth to a moderate degree. On the other hand, *F. oxysporum* and *F. redolens* were nonpathogenic to both seedling and post-seedling Marquis wheat plants. In both experiments, the intensity of root infection was negatively associated with plant growth and yield.

TABLE 3.—*The relative pathogenicity to seedling and adult Marquis wheat plants of different species of Fusarium*

(Data are means of 2 trials)

Organism	Seedling test			Adult plant test		
	Plant emergence	Disease rating	Green weight	Disease rating	Green weight	Grain yield
	Per cent		*Grams*		*Grams*	*Grams*
F. culmorum	40.1	88.0	9.7	59.0	88.4	11.1
F. avenaceum	79.6	9.5	62.8	32.4	113.2	15.0
F. equiseti	78.0	6.5	63.9	26.8	124.4	16.7
F. oxysporum	81.6	5.2	64.8	18.8	119.8	18.7
F. redolens	82.3	4.6	63.8	15.6	125.6	19.2
Control	82.2	4.8	65.2	13.6	126.1	22.3
Necessary difference, 5% level	9.9	12.9	10.1	11.2	12.6	6.1

Field Experiment. A field experiment was made in 1938 to determine the relative pathogenic capabilities of *Fusarium avenaceum, F. equiseti, F. oxysporum, F. redolens,* and 9 isolates of *F. culmorum* to adult wheat plants. The isolates of *F. culmorum* used gave, when tested under greenhouse conditions, a wide range in degrees of virulence on wheat seedlings (Table 2). For the field tests, different seed lots of wheat were inoculated with the respective isolates and fungi by the spore-suspension method. Immediately after inoculation the seed was planted in plots of artificially infested soil.

The analyses of variance for the plant emergence, disease, and yield data

TABLE 4.—*Analysis of variance for plant emergence, disease rating and yield. (Winnipeg Field Experiment, 1938)*

Source of variance	Degrees of freedom	Mean square		
		Plant emergence	Disease rating	Yield
Replicates	3	535.19[a]	149.24[a]	146.44[a]
Organisms	15	983.26[a]	54.96[b]	108.91[a]
Error	45	38.28	25.44	20.45

[a] Exceeds mean square error, 1% level of significance.
[b] Exceeds mean square error, 5% level of significance.

of the experiment are given in table 4. It is evident from this table that highly significant differences between organisms were obtained for all properties measured.

TABLE 5.—*Relative pathogenicity of five species of Fusarium, and of several isolates of Fusarium culmorum to Renown wheat under field conditions in 1938*

(Data are means of 4 plots)

Organism	Isolate No.	Percentage of plants emerged	Disease rating	Yield per acre (bushels)
F. equiseti	94	62.4	39.0	32.7
F. avenaceum	66	52.2	38.6	35.0
F. redolens	26	64.7	36.9	34.6
F. oxysporum	73	61.3	36.7	36.3
F. culmorum	937	37.5	43.4	28.4
	19A	28.6	43.2	27.4
	529	30.8	41.4	24.2
	1263	28.8	41.1	22.4
	38	39.3	41.0	25.0
	285	34.1	40.3	26.3
	10E	35.3	39.2	26.5
	19B	53.1	38.0	26.3
	15	49.8	36.9	24.3
	19C	48.2	34.6	34.1
Control—A	....	66.6	31.0	36.9
Control—B	....	67.1	30.9	38.1
Necessary difference, 5% level	....	9.3	7.6	6.8

In table 5 are given field-plot data for the different organisms. It is evident that, of the species tested, *Fusarium avenaceum* and *F. equiseti* were only slightly pathogenic to adult plants of Renown wheat, while the amount of infection on plants inoculated with *F. redolens* and *F. oxysporum* was not significantly different from that on the uninoculated control plants. The field results with *F. avenaceum, F. equiseti, F. redolens* and *F. oxysporum* were in accordance with the greenhouse results with these fungi (Table 3). On the other hand, the field results with different isolates of *F. culmorum* were not in agreement with the earlier greenhouse tests (Table 2). For instance, some of the isolates that proved to be distinctly pathogenic to post-seedling plants in the field were only weak parasites on wheat seedlings under greenhouse conditions, while others that showed a very low degree of virulence on adult plants in the field were strongly pathogenic to young wheat plants in the greenhouse tests. Further studies are required to determine the nature and cause of the variability observed in the parasitic behavior of different isolates of this important cereal root-infecting fungus.

Influence of Soil Moisture and Temperature on the Pathogenicity of Fusarium Species

Greenhouse pot experiments were conducted to determine the effect of soil moisture and soil temperature on the pathogenicity of *Fusarium culmorum* (Isolate 38), *F. equiseti, F. avenaceum, F. redolens,* and *F. oxysporum.*

Soil Moisture. Two experiments were made to determine the influence of moisture on pathogenicity. At the commencement of each experiment the soil in one half of the pots of each fungus series was adjusted to 60 per cent of its moisture-holding capacity (high moisture series), while the soil in the remainder of the pots was adjusted to a moisture-holding capacity of 40 per cent (low moisture series). High and low soil moisture pots of each fungus series were placed in each of 4 thermostatically controlled temperature tanks maintained at 10, 15, 20, and 25 degrees C., respectively. The respective soil moistures were maintained throughout the experiment.

The results of both soil-moisture experiments are summarized in table 6. To economize space, the complete analyses of variance for plant emergence, disease rating, and green weight of plants are not given. For each experiment, however, the results of these analyses showed that there were no moisture effects. The interactions of moistures and temperatures, and moistures and organisms, were also insignificant. On the other hand, significant differences between organisms were shown for all properties studied. In both experiments, temperature effects were significant for disease rating and green weight, but not for plant emergence.

TABLE 6.—*The influence of soil moisture on the pathogenicity of species of Fusarium to Marquis wheat seedlings (Data are means of four different temperatures—10, 15, 20, 25° C., and of 2 trials)*

Experiment	Organism	Percentage of plants emerged		Disease rating		Green weight of plants (grams)	
		Moisture content of soil[a]					
		High	Low	High	Low	High	Low
I	*F. culmorum*	37	40	85.7	88.1	6.7	8.8
	F. equiseti	47	48	3.8	5.8	50.8	52.4
	F. avenaceum	48	48	9.0	9.3	49.8	54.0
	Control	50	52	4.3	4.8	50.1	50.1
	Mean	45.5	47.0	25.7	25.8	39.4	41.3
II	*F. culmorum*	34	30	81.4	88.4	3.8	2.8
	F. redolens	54	54	13.6	6.6	27.8	29.0
	F. oxysporum	51	51	12.4	7.5	27.3	28.3
	Control	53	52	9.4	6.2	27.9	29.2
	Mean	48.0	46.8	29.2	27.2	22.0	22.3

[a] Differences between soil-moisture contents insignificant statistically.

The evidence presented in table 6 shows that soil moisture did not significantly increase or decrease the virulence of any of the species of *Fusarium* studied. In experiments I and II, however, there was a general tendency for low soil moisture to increase the virulence of these fungi. The results in table 6 show that *F. culmorum* (Isolate 38) was exceedingly pathogenic to Marquis wheat seedlings at both the low and high soil-moisture contents.

Soil Temperature. To ascertain the influence of temperature on the pathogenicity of different species of *Fusarium,* 2 experiments, each consisting of 3 organisms and a control, were carried out. The fungus *F. culmorum* was included in both experiments, and each experiment was run at 4 different times. The soil for each fungus series was inoculated in the usual manner and placed in sixteen 6-inch pots. Four pots of this soil were planted with 120 inoculated seeds of Marquis wheat and placed in each of 4 temperature tanks maintained at 10, 15, 20, and 25 degrees C., respectively.

The disease rating and green-weight data of the soil-temperature experiments are given in tables 7 and 8. These tables also give the temperature data recorded for the low-moisture series of the above-mentioned soil moisture experiments.

TABLE 7.—*The influence of temperature on the pathogenicity of different species of Fusarium to Marquis wheat seedlings. Results of Soil Temperature Experiments I and II, and Soil Moisture Experiments I and II*

Degree of Seedling Infection (Disease Rating)

Experiment	Organism	Temperature ° C.				Necessary difference between temperatures[a]
		10°	15°	20°	25°	
Temperature I[b]	*F. culmorum*	72.5	87.4	88.0	89.2	6.8
	F. equiseti	2.4	4.6	7.8	4.1	
	F. avenaceum	5.1	7.8	9.5	14.3	
	Control	2.8	4.1	4.8	7.4	
	Mean	20.7	26.0	27.2	29.2	3.4
Temperature II[b]	*F. culmorum*	78.9	88.4	83.8	84.3	9.9
	F. redolens	1.4	9.3	11.2	16.4	
	F. oxysporum	1.5	7.4	14.1	16.0	
	Control	1.3	6.9	11.8	12.1	
	Mean	20.8	28.0	30.2	32.2	5.0
Moisture I[c]	*F. culmorum*	66.2	87.5	89.4	90.2	6.6
	F. equiseti	2.2	4.2	8.1	8.5	
	F. avenaceum	2.9	7.0	10.0	17.4	
	Control	2.2	3.6	5.1	8.2	
	Mean	18.4	25.6	28.2	31.1	3.3
Moisture II[c]	*F. culmorum*	86.4	88.8	92.4	86.0	11.7
	F. redolens	1.2	5.2	6.7	13.2	
	F. oxysporum	1.4	4.4	11.6	12.5	
	Control	0.8	6.2	7.3	10.6	
	Mean	22.2	26.2	29.5	30.6	5.8

Necessary difference, 5% level of significance, between organisms: Temperature Experiment I = 6.8; Temperature Experiment II = 9.9; Moisture Experiment I = 6.6; Moisture Experiment II = 11.7.

[a] 5% level of significance.

[b] Data are means of 4 trials.

[c] Data are means of low soil moisture series, and of 2 trials.

The analysis of variance results of the data of each temperature and moisture experiment established that the effects of temperature on the pathogenicity of the species of *Fusarium* tested were very great. Although temperature did not influence significantly the percentage of plants that emerged from the soil, it had a very significant effect on the incidence of disease, as represented by the disease rating, and on the resulting green weight of the plants. In these experiments, as in previous pathogenicity tests, highly significant differences in the percentage of plants emerged, amount of disease, and total green weight of plants were observed between the species of *Fusarium* tested. There were no interactions between temperatures and organisms in any of the experiments.

TABLE 8.—*The influence of temperature on the pathogenicity of different species of Fusarium to Marquis wheat seedlings. Results of Soil Temperature Experiments I and II, and Soil Moisture Experiments I and II*

Green Weight of Plants (Grams)

Experiment	Organism	Temperature ° C.				Necessary difference between temperatures[a]
		10°	15°	20°	25°	
Temperature I[b]	*F. culmorum*	6.1	7.1	9.7	8.1	18.6
	F. equiseti	29.0	51.6	63.9	62.0	
	F. avenaceum	29.6	55.4	62.8	59.8	
	Control	30.5	50.1	65.2	59.6	
	Mean	23.8	40.1	50.4	47.4	9.3
Temperature II[b]	*F. culmorum*	4.6	2.6	4.4	3.6	8.6
	F. redolens	22.6	34.5	31.3	25.1	
	F. oxysporum	20.7	33.2	32.4	24.9	
	Control	23.1	32.7	32.8	25.7	
	Mean	17.8	25.8	25.2	19.8	4.3
Moisture I[c]	*F. culmorum*	9.5	9.3	9.1	7.4	21.6
	F. equiseti	32.4	54.2	65.1	57.8	
	F. avenaceum	34.0	60.0	66.6	55.4	
	Control	33.8	53.6	67.0	56.1	
	Mean	27.4	44.3	52.0	44.2	10.8
Moisture II[c]	*F. culmorum*	4.1	2.3	1.8	3.1	9.2
	F. redolens	22.5	35.2	36.3	21.9	
	F. oxysporum	21.1	35.2	35.0	22.0	
	Control	23.3	33.5	36.0	24.2	
	Mean	17.8	26.6	27.3	17.8	4.6

Necessary difference, 5% level of significance, between organisms: Temperature Experiment I = 18.6; Temperature Experiment II = 8.6; Moisture Experiment I = 21.6; Moisture Experiment II = 9.2.

[a] 5% level of significance.

[b] Data are means of 4 trials.

[c] Data are means of low soil moisture series, and of 2 trials.

From table 7 it is evident that temperature had a very marked effect on the virulence of the species of *Fusarium* studied. The mean-temperature figures for organisms indicate that, in all experiments, the greatest amount of injury to Marquis wheat seedlings occurred at the higher temperatures, that is, at 20° and 25° C. On the other hand, plant growth was considerably more vigorous at 20° C. than at 25° C. (Table 8). Thus, it may well be that the effect of temperature on the host is more important than its effect on the fungus. The extensive investigations of Dickson (8), McKinney

(19), and others, have indicated that young wheat plants are blighted most severely by root-rotting fungi at temperatures that serve to predispose the plants to disease, that is, relatively high temperatures for wheat. The evidence presented in tables 7 and 8 substantiates the conclusions of Dickson and McKinney, and indicates that temperature is an exceedingly important factor influencing the occurrence of seedling blight and root rot of wheat caused by *Fusarium* species.

Influence of Other Soil-Inhabiting Fungi on the Pathogenicity of Fusarium Species

Two greenhouse pot experiments were made to determine the effects of the association of certain common soil-inhabiting fungi on the pathogenicity of *Fusarium redolens,* a very weak pathogen, and on a virulent isolate (No. 38) of *F. culmorum* (Table 3). In these experiments, wherever association effects were studied, the total quantity of inoculum incorporated into the soil was double the amount used where the fungus was tested singly. The common soil-inhabiting fungi employed were *Pyronema confluens* (Per.) Tul., *Trichoderma lignorum* (Tode) Harz, *Aspergillus flavipes* (B. and S.) Thom. and Church, and *Penicillium intricatum* Thom. These fungi were originally isolated from the soil of permanent grain plots at Winnipeg, Man. In experiment I, *P. confluens* and *T. lignorum* were tested against *F. culmorum,* while *A. flavipes* and *P. intricatum* were tested against *F. culmorum* and *F. redolens* in experiment II.

In experiments I and II, 4 pots of each fungus, or combination of fungi, were prepared, planted, and placed in temperature tanks maintained at 10° and 20° C. The soil-moisture content of each pot was held at 50 per cent of the total water-holding capacity of the soil throughout the experiment. The plants were harvested at 28 days, and the disease and yield data recorded in the usual way. An adequate number of control pots were included in each experiment, and both experiments were repeated 4 times.

The analyses of variance results of both experiments showed that a high degree of significance could be attached to the differences observed in percentage of plants emerged, amount of disease, and yield, between the various organisms and combinations of organisms studied in each experiment. As might be expected from the results of the temperature experiments described in the preceding section, significant temperature effects were shown for both disease rating and green weight of the plants, but not for plant emergence.

The summarized results in table 9 show the marked virulence of *Fusarium culmorum* (Isolate 38), and the weak parasitic action of *F. redolens,* on wheat seedlings. The important point in these experiments is the fact that the presence of the fungi *Pyronema confluens* and *Trichoderma lignorum* in soil, suppressed, to an appreciable degree, particularly at 10° C., the virulence of *F. culmorum.* On the other hand, the presence of *Penicillium intricatum* and *Aspergillus flavipes* in the soil had no influence on the pathogenicity of *F. culmorum*. The pathogenicity of *F. redolens* was not influenced by the presence of *P. intricatum* and *A. flavipes* in the soil.

The results of Experiments I and II indicate the marked effect temperature has on the pathogenicity of species of *Fusarium*. In both experiments, *F. culmorum*, whether alone or in association with other soil-inhabiting fungi, was much more virulent at 20° C. than at 10° C.

TABLE 9.—*The effect of certain common soil-inhibiting fungi on the pathogenicity of Fusarium culmorum and Fusarium redolens to Marquis wheat seedlings*

(Data are means of 4 trials)

Organisms added to soil	Percentage of plants emerged		Disease rating		Green weight of plants (grams)	
	10°	20°	10°	20°	10°	20°
Experiment I						
Fusarium culmorum	17	16	86.1	91.3	1.6	4.4
Pyronema confluens	48	50	3.4	2.9	28.5	73.6
Trichoderma lignorum	48	48	2.8	3.3	25.6	81.1
F. culmorum + P. confluens	28	21	73.6	84.1	3.2	6.0
F. culmorum + T. lignorum	25	23	77.7	87.0	3.3	2.3
P. confluens + T. lignorum	48	48	2.4	2.4	27.7	66.5
Control	49	48	1.9	2.2	26.2	88.5
Necessary difference, 5% level	9.6	9.6	8.0	8.0	12.6	12.6
Temperature mean	37.6	36.3	35.4	39.0	16.6	46.1
Necessary difference, 5% level, between temperature means	a		3.0		4.8	
Experiment II						
Fusarium culmorum	28	19	59.1	88.3	4.7	2.8
Fusarium redolens	51	49	1.2	2.6	15.5	34.6
Aspergillus flavipes	51	52	1.6	2.5	14.4	36.0
Penicillium intricatum	46	50	1.0	3.3	13.6	37.1
F. culmorum + A. flavipes	24	20	61.2	86.0	3.7	3.5
F. culmorum + P. intricatum	28	19	61.0	89.6	4.2	3.0
F. redolens + A. flavipes	50	50	0.6	1.9	13.2	36.5
F. redolens + P. intricatum	52	49	0.7	2.3	14.0	36.2
Control	51	50	0.9	1.9	14.4	34.2
Necessary difference, 5% level	12.8	12.8	9.8	9.8	9.5	9.5
Temperature mean	42.3	39.8	20.8	30.9	10.8	24.8
Necessary difference, 5% level, between temperature means	a		3.3		3.2	

[a] Difference insignificant statistically.

In general, the data presented in table 9 emphasize the importance of the reaction of one fungus upon another in the soil, and confirm the experiments of Henry (18), Sanford and Broadfoot (25), Garrett (10), Bisby, James, and Timonin (5), and others, who found that infection of wheat seedlings by cereal root-rotting fungi was suppressed by the antagonistic effect of certain other soil fungi and bacteria.

DISCUSSION

In this paper additional evidence is provided to support the view that certain common soil-inhabiting fungi have a marked influence on the development of cereal root-rot fungi. It was found that infection of wheat seedlings with a virulent isolate of *Fusarium culmorum* alone, or in association with certain other common soil-inhabiting fungi, increased with a rise in temperature from 10° to 20° C. However, the conclusions of Garrett (9) and others suggest that the results observed in the present association studies represent not the true effect of temperature upon infection with *F. culmorum,* but a combination of this with the action of temperature upon biological antagonism of the soil microflora. In the present studies the antagonism of *Pyronema confluens* and *Trichoderma lignorum* to *F. culmorum* was greater at 10° than at 20° C. The work of Garrett (9), however, has shown that the antagonism of other soil bacteria and fungi to certain root-rot fungi of cereals increases with a rise in temperature to 30° C. In view of these conflicting results it is apparent that more detailed experiments are needed to determine the true effect of soil temperature upon the antagonism of other soil fungi to the root-rot fungi of cereals.

The present studies have demonstrated that the seedling stage in wheat is a very critical one from the standpoint of root rot caused by species of *Fusarium.* The primary roots of young wheat plants appear to be very susceptible to invasion and injury by virulent strains of these fungi. These experiments have further shown that the onset and progress of the Fusarium disease is greatly enhanced by weakened plant growth. Thus, the data provided by the investigation are considered sufficient to justify the conclusion that any farm practice that will stimulate early, rapid, and vigorous plant growth will be an effective means of reducing the losses due to seedling blight and root rot of wheat caused by species of *Fusarium.*

SUMMARY

Greenhouse experiments were made to determine the pathogenicity to wheat of 24 isolates of *Fusarium culmorum* (W. G. Sm.) Sacc., and of 4 other species of *Fusarium,* namely, *F. redolens* Wr., *F. oxysporum* Schl. v. *aurantiacum* (Lk.) Wr., *F. avenaceum* (Fr.) Sacc. and *F. equiseti* (Cda.) Sacc. The species of *Fusarium* studied were those most commonly isolated from root-rotted specimens of wheat, and from grain soils collected in Manitoba. Of the isolates of *F. culmorum* tested only one was distinctly pathogenic to both seedling and adult wheat plants. Under greenhouse conditions, no marked pathogenicity was exhibited by the isolates of *F. redolens, F. oxysporum, F. avenaceum* and *F. equiseti* studied. Field tests with these fungi gave essentially similar results.

The influence of soil moisture and soil temperature on the virulence of different species of *Fusarium* on wheat seedlings was studied by means of greenhouse pot tests. The moisture content of the soil did not influence significantly the virulence of the *Fusarium* species tested. On the other hand, intensity of infection with these fungi increased with an increase in soil temperature. The isolate of *F. culmorum* studied was distinctly pathogenic at 10, 15, 20, and 25° C. *Fusarium avenaceum* was pathogenic, but

weakly so, only at 25° C., while *F. redolens*, *F. oxysporum* and *F. equiseti* were not pathogenic at any of the temperatures employed.

In greenhouse pot tests, *Trichoderma lignorum* Tode (Harz.) and *Pyronema confluens* (Pers.) Tul., two common soil inhabitants, suppressed, to an appreciable degree, the pathogenicity of a virulent isolate of *F. culmorum*, while *Penicillium intricatum* Thom, and *Aspergillus flavipes* (B. and S.) Thom and Church, did not. The virulence of *F. redolens* was not influenced by the presence of *P. intricatum* and *A. flavipes* in the soil.

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CROSS INOCULATIONS WITH ISOLATES OF FUSARIA FROM COTTON, TOBACCO, AND CERTAIN OTHER PLANTS SUBJECT TO WILT[1]

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INTRODUCTION

Burley tobacco, *Nicotiana tabacum* L., was grown for the first time in 1938 in several counties of the Piedmont section of South Carolina, and wilting occurred in several fields. Tissue cultures of affected plants invariably gave a species of *Fusarium*. Since there was no previous record that tobacco had been grown in these fields and, since cotton, *Gossypium hirsutum* L., was known to wilt in most of them, cross-inoculation experiments were undertaken with the Fusaria from these hosts.

Wilt of coffeeweed or wild senna (*Cassia tora* L.[2]) was noted in an experimental cotton wilt plot at Allendale, South Carolina, in 1937 and similar plants were later found at 2 other locations in the State. Isolations from plants collected at all these locations yielded Fusaria similar in cultural appearances. These observations suggested that the cotton wilt Fusarium, besides existing as a saprophyte in the soil, might attack weeds and thus be perpetuated in a living host other than cotton. Therefore, cross inoculations with these Fusaria were made.

Several strains of wilt-resistant cowpeas, *Vigna sinensis* Endl., were grown on a plot known to be heavily infested with the cotton-wilt organism, but the presence of the cowpea-wilt organism in this soil had not been established. The nonresistant cowpeas used as checks were severely damaged by wilt, consequently cross inoculations of cotton and cowpeas with the fusarium-wilt organisms from these hosts were included in the present studies.

Several early publications refer to *Fusarium vasinfectum* Atk. as the cause of wilt of okra, *Hibiscus esculentus* L., without presenting adequate experimental evidence to support these statements.[3] Hence, okra and its fusarium isolate were also used in these experiments. Since wilts of tomato and watermelon are generally distributed throughout the State, tomato (*Lycopersicon esculentum* Mill.) and watermelon (*Citrullus vulgaris* Schrad.) and their fusarium isolates were included in the experiments after it had been discovered that the cotton-wilt fungus causes wilt in several other plants.

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[1] Contribution of the Department of Botany and Bacteriology in cooperation with the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Technical contribution No. 86, South Carolina Agricultural Experiment Station.

[2] *Emelista tora* (L.) Britton and Rose, according to Small.

[3] After this paper was submitted for publication, it was discovered that Taubenhaus had performed cross inoculations with wilt Fusaria from cotton, okra, cowpeas, watermelon, tomato, cabbage, squash, and sweetpea. Taubenhaus, J. J. Wilts of watermelon and related crops. Tex. Agr. Exp. Stat. Bull. 260, 50 pp. 1920.

METHODS AND MATERIALS

The solution-culture infection technique (3) employed in other studies of cotton wilt was used chiefly in these studies. As a check on the solution-culture method, tobacco and cotton also were grown in pots of soil, each inoculated with a wheat-oats mixture of the fungal isolates from one of the several hosts. The procedure was the same as that described in other experiments by the senior writer and associates (2). The isolate of the fungus from cotton had been used in other tests and was known to be strongly pathogenic. In addition to the fusarium culture isolated from tobacco in South Carolina, 1 from Kentucky[4] and 3 from Maryland[5] also were used in the experiments. A susceptible and a resistant variety of Burley tobacco, Burley 5 and Burley 31, respectively, and the Gold Dollar variety of flue-cured tobacco were grown, but only the susceptible Farm Relief 2 variety of cotton was used. All flue-cured varieties of tobacco grown in South Carolina are apparently highly resistant to the fusarium-wilt organism in the soils of the State, since the disease is seldom seen in the fields. The cultures of the Cassia-, cowpea-, okra-, and watermelon-wilt organisms were obtained in South Carolina.

For the solution-culture method, all seed, except that of tobacco, was placed directly in the excelsior seed bed of the trays, as previously described (3). This procedure was impossible with the small seed of tobacco, so these were planted in flats of steamed soil. When the seedlings were 4 to 6 inches tall they were transferred to both soil and solution cultures. For inoculations of tobacco in soil, 2 plants were grown in each 2-gal. glazed pot, the transplanting being done 24 days after the wheat-oats inoculum was mixed with the soil.

Plants were removed from trays or pots as wilting occurred. Approximately an inch section was cut from the base of each stem, sterilized in a 3 per cent solution of sodium hypochlorite[6] for 5 minutes, and plated on 2 per cent potato dextrose agar. At the end of an experiment all remaining plants were treated similarly.

EXPERIMENTAL RESULTS

Cross Inoculations of Cotton and Cassia tora

The first seed of Cassia was collected early in the fall from plants showing symptoms of wilt injury; but the fact that these had survived to seed production indicated a resistance to the wilt fungus. The second lot of seed was gathered late in the fall probably after the seed from wilt-injured plants had been shed, and at a time when only the vigorous, resistant, and later-maturing plants were available. The very low percentages of infection from plants of the second lot (Table 7) indicate that these were characterized by very high wilt resistance. Observations in the field also support the idea that most plants are very resistant, since only a small percentage of stems showed internal blackening at the base. Thus in heavily infested soil it may be that natural selection has eliminated the susceptible plants. A year later a third collection of seed was made earlier in the season from plants showing very definite external symptoms of wilt, and the plants produced from this seed were the most wilt-susceptible of the 3 lots (Table 1, exp. 3).

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[4] Cultures supplied by W. D. Valleau.

[5] Cultures supplied by E. A. Walker.

[6] The commercial product, BK, was used.

TABLE 1.—*Inoculations of Cassia tora and cotton in solution cultures with each of 3 Fusaria*

Inoculated with	No. plants	Per cent plants with symptoms of wilt		Recovery of fungus, per cent
		External	Internal	
Cassia tora				
Cassia fungus (exp. 1)	169	20.7	33.7	30.8
S. C. cotton fungus (exp. 1)	175	3.4	6.8	17.1
S. C. tobacco fungus (exp. 1)	166	6.0	12.6	24.7
S. C. tobacco fungus (exp. 3)	88	42.0	42.0	22.7
Uninoculated check (exp. 1)	96	0.0	7.3	9.4
Uninoculated check (exp. 3)	91	0.0	0.0	2.2
Cotton, Farm Relief 2				
Cassia fungus (exp. 1)	137	27.0	24.1	31.4
Cassia fungus (exp. 3)	78	98.7	98.7	82.0
S. C. cotton fungus (exp. 1)	150	88.0	86.7	82.0
S. C. tobacco fungus (exp. 1)	142	92.2	91.5	88.0
Uninoculated check (exp. 1)	64	6.2	10.9	17.2
Uninoculated check (exp. 3)	80	0.0	0.0	1.2

That *Cassia tora* is subject to infection by a species of *Fusarium* obtained from this host seems evident from the results reported in table 1, exp. 1. The percentages of infection, whether based on external symptoms, internal symptoms, or isolations of a *Fusarium,* are not high but are in the expected range for a resistant variety. Such low percentages of infection were obtained in the second experiment (Table 7) that very definite statements are not warranted from these results. As indicated above, however, the seed for this experiment probably was collected from highly resistant plants. There was slight internal blackening of a few plants and fusaria were obtained similar in appearance to the original cultures.

The results presented in table 1 indicate that *Cassia tora* is also subject to infection by the wilt fungi from cotton and tobacco. Since the percentages of infection were low in the first 2 tests, a third collection of Cassia seed was made. It will be seen in table 1, exp. 3, that 42 per cent of the plants inoculated with the South Carolina tobacco fungus showed external symptoms of wilt. The plants from which this collection of Cassia seed was made produced seed less abundantly than nearby healthy plants, but the fact that the diseased plants survived would place them in a class resistant to wilt.

It should be noted that if infection experiments on Cassia with the fungi from cotton and Cassia had been confined to the plants derived from the second seed lot, the conclusion would have been that this weed is probably not a host for the cotton-wilt fungus (Table 7). On the other hand, the results obtained with the third collection of seed (Table 1, exp. 3) would indicate that this weed is more highly susceptible to wilt caused by the *Fusarium* species common to it and cotton and tobacco in South Carolina than field observations show.

Attention is called to the infection that occurred in the check trays of Cassia and cotton (Table 1). Elliott (8), Taubenhaus and Ezekiel (17), Crawford (7), and Kulkarni (12) have stated that some of the seed from cotton plants infected with the fusarium-wilt organism may carry the fungus internally. Nothing is known about the presence of the fungus internally in *Cassia tora,* but this is a possibility, since the seed used in the first and third experiments (Table 1) came from wilting plants. However, not all the infection in the checks of the first experiments may have come from this source, since experience with the solution-culture method has indicated that accidental infections can be avoided only by meticulous care in every detail of handling the cultures. It is believed that the weekly changing of the solution in which the trays were lifted from the bench and the solution emptied into a sink was the chief cause of trouble. Fewer infections occurred in the checks when the technique was gradually improved, so that trays were not handled, the solution was drained through sterilized syphons, new solution was added through sterilized glass tubes, and sterilized rubber gloves were worn and changed after handling the cultures inoculated with any single isolate.

Experience gained in culturing many thousands of plants indicated that a Fusarium could not always be recovered from a darkened stem, but one might be obtained from a fairly high percentage of plants of resistant varieties that show neither external nor internal symptoms of wilt. In many of the severe cases of wilting, the plants were all but dead before they were removed and cultured. In such cases fast-growing contaminating organisms made the recovery of the Fusarium difficult.

It is recognized, also, that obtaining a Fusarium from a stalk is no absolute assurance that the wilt fungus has been recovered, even though the isolate be similar in appearance to the parent culture. Tests would be necessary to prove its pathogenicity, but the great amount of labor and equipment involved make this procedure impracticable. However, those tests that have been made with a limited number of isolations indicated that the error from this source was small. *Fusarium moniliforme* frequently is encountered if a dozen or more stalks are being cultured; but its growth characteristics are distinct from the wilt-producing forms, thus usually making unnecessary any microscopic examinations to distinguish this organism. In one check solution culture, 36 per cent of the stalks gave *F. moniliforme,* but there was no wilt, and the plants were growing vigorously.

That cotton is subject to infection by the fusarium-wilt organism obtained from *Cassia tora* is evident from the results presented in tables 1, 2, and 7. In the solution-culture experiments, 27 per cent (Table 1, exp. 1), 98.7 per cent (Table 1, exp. 3) and 100 per cent (Table 7) of the plants showed external symptoms of wilt. In a soil-culture experiment (Table 2), 100 per cent of the plants also showed symptoms of wilt. The Cassia fungus also caused wilt in the susceptible Burley tobacco (Table 2). This fungus has not been used in an infection experiment with okra, but successful infections would be expected. Smith (14) reported that . . . "Mr. Orton has found the fungus on James Island, South Carolina, in a weed, the *Cassia obtusifolia* L.[7]" and that the external and internal symptoms of wilt were identical with those on cotton and cowpea. The observations were not supported by experimental evidence as to the connection of "the fungus" with the disease.

TABLE 2.—*Inoculations of cotton and tobacco in soil with the Fusarium from Cassia tora*

Inoculated with	No. plants	Per cent plants with symptoms of wilt		Recovery of fungus, per cent
		External	Internal	
Cotton, Farm Relief 2				
Cassia fungus	31	100.0	100.0	100.0
Uninoculated check	9	11.1	11.1	11.1
Tobacco, Burley 5				
Cassia fungus	31	87.1	87.1	96.8
Uninoculated check	8	25.0	25.0	50.0

Cross Inoculations of Cotton and Tobacco

In these experiments cotton has been inoculated in solution culture only, with Fusaria isolated from cotton and tobacco in South Carolina, from tobacco in Kentucky, and 3 cultures from tobacco in Maryland. Other experiments (2) have shown that several cotton isolates gave the same relative rating as to pathogenicity for cotton in solution culture as in soil, but higher percentages of wilting have occurred in the former.

TABLE 3.—*Inoculation of cotton in solution cultures with the fusarium-wilt fungus of tobacco from Kentucky*

Inoculated with	No. plants	Per cent plants with symptoms of wilt		Recovery of fungus, per cent
		External	Internal	
Cotton, Farm Relief 2				
Ky. 627 tobacco fungus	281	91.8	90.4	59.8
Uninoculated check	100	0.0	2.0	2.0

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[7] Synonym of *Cassia tora* L.

The data of tables 1 and 3 show that the susceptibile Farm Relief variety of cotton inoculated with the South Carolina cotton fungus, the South Carolina tobacco fungus, and the Kentucky 627 tobacco fungus showed external symptoms of wilt in the inoculated plants of 88, 92.2, and 91.8 per cent, respectively. The same variety of cotton, inoculated with the South Carolina cotton fungus at a later date, showed 100 per cent of the plants with external symptoms of wilt (Table 7). The isolates of Fusarium from tobacco grown in South Carolina and Kentucky clearly produce wilting of cotton as readily as a virulent isolate from cotton. The tobacco isolates from Maryland, however, failed to infect the Farm Relief variety of cotton, as is noted below.

Tables 4 and 5 present the data obtained from inoculating the tobacco varieties, susceptible Burley 5, resistant Burley 31, and flue-cured Gold Dollar with 1 cotton- and 2 tobacco-fusarium isolates. Burley 5 inoculated in solution culture with the South Carolina cotton fungus, with the South Carolina tobacco fungus, and with the Kentucky 627 tobacco fungus showed external symptoms of wilt in 97.8, 88.9, and 78.7 per cent of the plants, respectively, (Table 4). Much smaller percentages of the plants of resistant varieties showed external symptoms of wilt, though the fungus was recovered from 56.5 per cent of Gold Dollar plants inoculated with the South Carolina tobacco fungus.

TABLE 4.—*Inoculations of 3 varieties of tobacco in solution cultures with each of 3 Fusaria*

Inoculated with	No. plants	Per cent plants with symptoms of wilt		Recovery of fungus, per cent
		External	Internal	
Tobacco, Burley 31				
S. C. cotton fungus	48	16.7	33.3	25.0
S. C. tobacco fungus	53	3.8	5.7	18.9
Ky. 627 tobacco fungus	48	12.5	12.5	6.2
Uninoculated check	24	8.3	8.3	12.5
Tobacco, Burley 5				
S. C. cotton fungus	45	97.8	100.0	88.9
S. C. tobacco fungus	45	88.9	93.3	66.7
Ky. 627 tobacco fungus	47	78.7	85.1	42.5
Uninoculated check	23	13.0	13.0	8.7
Tobacco, Gold Dollar				
S. C. cotton fungus	46	13.0	23.9	63.0
S. C. tobacco fungus	46	0.0	21.7	56.5
Ky. 627 tobacco fungus	48	6.3	6.3	29.1
Uninoculated check	24	16.7	16.7	16.7

Data obtained from a similar series of inoculations in soil are given in table 5. Plants of Burley 5 were the only ones to show any external symptoms of wilt, though the fungus was isolated from 37.1 per cent of the Gold Dollar plants inoculated with the South Carolina tobacco fungus. The first external symptoms of wilt in Burley 5 plants appeared 19 days after transplanting to the soil, while, in the culture solution, only 10 days elapsed after inoculation before wilt symptoms were noted. There was more rapid wilting and a considerably higher percentage of infection in the solution cultures than in the soil.

It is evident that wilt in a susceptible Burley tobacco can be produced by inoculations with the Fusaria causing wilt in cotton and in tobacco. The percentages of Burley 5 plants showing external symptoms of wilt with the Kentucky 627 tobacco fungus were less in both soil and solution than with the other isolates. It is probably incorrect to conclude, however, that the Kentucky isolate is less pathogenic than the others, since these percentages are based on a small number of plants. The data of tables 1 and 3 showing the results of the inoculation of a larger number of cotton plants do not indicate such differences. Several months after these experiments were completed, 3 cultures of tobacco-wilt Fusaria from Maryland were obtained. The most virulent culture, "Mudd," was used to inoculate 79 plants of Farm Relief cotton, but only 2 plants showed any symptoms of wilt (Table 6, exp. 1). Since the seed might carry the fungus internally to an extent considerably greater than this, it appeared that the Maryland strain of the fungus was distinctly different from the strains obtained in South Carolina and Kentucky. Accordingly, the 3 Maryland isolates were used to inoculate a susceptible variety of cotton and the Burley 5 and Gold Dollar varieties of tobacco. None of the cotton plants showed any external or internal symptoms of wilt in this test (Table 6, exp. 2), but both varieties of tobacco proved susceptible to all the isolates. Gold Dollar had proved resistant to the South Carolina and Kentucky isolates, but showed 33.3 per cent external wilt with

TABLE 5.—*Inoculations of 3 varieties of tobacco in soil with each of 3 Fusaria*

Inoculated with	No. plants	Per cent plants with symptoms of wilt		Recovery of fungus, per cent
		External	Internal	
Tobacco, Burley 31				
S. C. cotton fungus	30	0.0	0.0	3.3
S. C. tobacco fungus	27	0.0	3.7	11.1
Ky. 627 tobacco fungus	35	0.0	0.0	17.1
Uninoculated check	16	0.0	0.0	0.0
Tobacco Burley 5				
S. C. cotton fungus	30	53.3	53.3	70.0
S. C. tobacco fungus	29	58.6	58.6	58.6
Ky. 627 tobacco fungus	31	38.7	41.9	71.0
Uninoculated check	15	0.0	0.0	0.0
Tobacco, Gold Dollar				
S. C. cotton fungus	30	0.0	0.0	23.3
S. C. tobacco fungus	35	0.0	2.9	37.1
Ky. 627 tobacco fungus	32	0.0	0.0	18.8
Uninoculated check	16	0.0	0.0	0.0

TABLE 6.—*Inoculations of cotton and tobacco in solution cultures with 3 isolates of Maryland tobacco-wilt Fusaria*

Inoculated with	No. plants	Per cent plants with symptoms of wilt		Recovery of fungus, per cent
		External	Internal	
Cotton, Farm Relief 2				
Maryland, "Mudd" (exp. 1)	79	2.5	2.5	11.4
Maryland, "Mudd" (exp. 2)	52	0.0	0.0	3.8
Maryland 18 (exp. 2)	62	0.0	0.0	3.2
Maryland 21 (exp. 2)	57	0.0	0.0	3.5
Uninoculated check (exp. 1)	69	0.0	0.0	1.4
Uninoculated check (exp. 2)	43	0.0	0.0	0.0
Tobacco, Burley 5				
Maryland, "Mudd"	67	97.0	97.0	97.0
Maryland 18	62	93.5	95.2	98.4
Maryland 21	63	96.8	98.4	100.0
Uninoculated check	34	0.0	17.6	17.6
Tobacco, Gold Dollar				
Maryland, "Mudd"	57	77.2	94.7	96.5
Maryland 18	54	53.7	94.4	92.6
Maryland 21	54	33.3	74.1	77.8
Uninoculated check	27	0.0	0.0	14.8

the least virulent Maryland isolate and 77.2 per cent wilt with the most virulent isolate.

TABLE 7.—*Inoculations of cotton, cowpea, and Cassia tora in solution cultures with each of 3 Fusaria*

Inoculated with	No. plants	Per cent plants with symptoms of wilt		Recovery of fungus, per cent
		External	Internal	
Cotton, Farm Relief 2				
S. C. cotton fungus	165	100.0	96.4	93.3
Cowpea fungus	158	0.63	1.3	7.0
Cassia fungus	143	100.0	95.8	96.5
Uninoculated check	74	0.0	4.1[a]	1.3
Cowpea, Cal. Blackeye				
S. C. cotton fungus	153	0.0	0.65	6.5
Cowpea fungus	141	100.0	97.2	95.7
Cassia fungus	152	0.66	2.0	8.6
Uninoculated check	78	0.0	0.0	2.6
Cassia tora				
S. C. cotton fungus	225	0.0	8.4	6.2
Cowpea fungus	207	0.48	1.4	8.2
Cassia fungus	225	0.0	4.0	12.4
Uninoculated check	110	0.0	0.0	0.0

[a] 3 plants with faint, darkened streaks.

Cross Inoculations of Cowpea, Cassia tora, and Cotton

The susceptible Farm Relief 2 cotton, the susceptible California blackeye cowpea, and an apparently highly resistant *Cassia tora* were inoculated in solution culture with wilt Fusaria from each of the host plants.

The data of table 7 indicate that the cowpea organism is quite distinct, and that the organisms from cotton and *Cassia tora* are the same. The few recoveries of Fusaria from cowpeas inoculated with the isolates from cotton and Cassia may have been due to accidental infections or the presence of the wilt fungus in or on the seed, since Kendrick (11) has shown that the fungus can be carried on the cowpea seed. It is possible also that the fungus may grow to some extent in old cortex, out of reach of surface sterilization. Smith (14) made numerous attempts to infect cotton, cowpeas, and watermelons with the wilt Fusaria from these hosts, but, except where the fungus was obtained from its specific host, no infection occurred. Johnson (10) failed to obtain infection of cowpeas from inoculations with a Maryland tobacco-wilt fungus. Carpenter (5) obtained no infection of Brabham cowpeas with an okra Fusarium.

Inoculations of Cotton, Okra, and Tobacco

Carpenter (5) inoculated 99 okra plants with the cotton-wilt fungus in 3 tests, and obtained only 1 wilted plant. He did, however, get 87 per cent wilt from the inoculation of 43 cotton plants with the okra fungus. Fahmy (9) failed to obtain infection of okra with the Egyptian cotton-wilt fungus.

The data of table 8 show clearly that the okra fungus will cause wilting

TABLE 8.—*Inoculations of okra, tobacco, and cotton in solution cultures with wilt Fusaria*

Inoculated with	No. plants	Per cent plants with symptoms of wilt		Recovery of fungus, per cent
		External	Internal	
Okra, Clemson Spineless				
Okra fungus (exp. 1)	80	91.2	100.0	93.8
Okra fungus (exp. 2)	84	100.0	100.0	83.3
S. C. cotton fungus	66	98.5	98.5	86.4
Maryland tobacco fungus "Mudd"	74	0.0	1.4	4.0
Uninoculated check (exp. 1)	41	0.0	0.0	0.0
Uninoculated check (exp. 2)	56	0.0	8.9	7.1
Tobacco, Burley 5				
Okra fungus	23	100.0	100.0	78.3
Uninoculated check	27	0.0	0.0	3.7
Cotton, Farm Relief 2				
Okra fungus	90	58.9	68.9	62.2
Uninoculated check	80	0.0	0.0	1.2

of okra, a susceptible Burley tobacco, and a susceptible cotton. The cotton fungus also caused wilting of okra. The "Mudd" isolate of the Maryland tobacco fungus, however, did not produce any visible wilt of okra.

Inoculations of Okra, Tomato, Cotton, and Watermelon

The data of table 9 show that the tomato was not attacked by the wilt fungi from okra or watermelon. Since the data from other experiments indicate that the wilt Fusaria from cotton, okra, Cassia, South Carolina tobacco, and Kentucky tobacco are the same, it seems reasonable to conclude that the tomato is not subject to wilt by any of these fungi. The tomato-wilt fungus[8] also did not produce wilt in watermelon, okra, or cotton. Bohn and Tucker (4) have indicated that the parasitism of *Fusarium lycopersici* is limited to the genus *Lycopersicon.*

TABLE 9.—*Inoculations of okra, tomato, cotton, and watermelon in solution cultures with wilt Fusaria*

Inoculated with	No. plants	Per cent plants with symptoms of wilt		Recovery of fungus, per cent
		External	Internal	
Tomato, Improved Earliana				
Tomato fungus	95	94.7	100.0	98.9
Okra fungus	91	0.0	1.1	5.5
Watermelon fungus	101	0.0	0.0	5.9
Uninoculated check	69	0.0	0.0	2.9
Watermelon, Florida Giant				
Watermelon fungus	49	100.0	100.0	95.9
Tomato fungus	45	0.0	0.0	2.2
S. C. cotton fungus	88	0.0	0.0	0.0
Uninoculated check	34	0.0	0.0	0.0
Okra, Clemson Spineless				
Tomato fungus	65	0.0	0.0	3.1
Uninoculated check	56	0.0	8.9	7.1
Cotton, Farm Relief 2				
Tomato fungus	59	0.0	0.0	0.0
Uninoculated check	43	0.0	0.0	0.0

The cotton fungus did not cause wilt of the watermelon, which agrees with the results obtained by Smith (14) many years ago.

DISCUSSION

The writers' experience with Fusaria has been confined largely to the section Elegans. It is generally recognized that the Fusaria may vary widely in the morphological and physiological characters, including pathogenicity, that are utilized in taxonomy. No attempt will be made to review the extensive literature on this subject, and only those references that bear directly on the present investigation will be cited.

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[8] Culture supplied by F. L. Wellman.

In earlier studies of cotton wilt by the senior writer, a large number of isolations of the fungus were made from wilting cotton stalks, chiefly from various sections of South Carolina. After these isolates had been in culture for different lengths of time, 19 cultures representing the range of cultural characteristics were selected for further study. When single-spore cultures were made of some of the isolates differing most in cultural characteristics, great variability was revealed (2). Variants appeared as early as the second transfer showed a tendency to dominate the parent culture. A series of variants, quite similar to the range of those reported for tomato wilt by Wellman and Blaisdell (18), were obtained. Some of the cotton cultures could not be identified as *Fusarium vasinfectum* according to descriptions given by Wollenweber and Reinking (19), yet they caused cotton wilt. Johnson (10) made cultural comparisons of isolates of *F. oxysporum* from potatoes with his fusarium cultures from tobacco and mentions the difficulties in making a decision as to their identity, since some of the potato cultures were "sub-normal," one in particular being less virulent than the vigorous mycelial types from tobacco. Only the vigorous mycelial types have been used for the inoculations reported in this paper, but one of the "sub-normal" types of the cotton-wilt fungus used in a previous study (2) proved as virulent as the vigorous mycelial types. Other "subnormal" or appressed cultures, however, were less virulent than most of the vigorous mycelial types, which agrees with the results of others studying several wilt Fusaria.

During the period from 1932 to 1935, inclusive, 4 attempts were made to differentiate culturally, 8 single-spore isolates of *Fusarium vasinfectum* by the technique of Coons and Strong (6), using the toxic malachite-green and crystal-violet dyes. Fairly distinct differences among several of the isolates were apparent in a single test, but repetitions of the experiment produced results inconsistent with those of the other tests. Since it has been found (2) that variants of *F. vasinfectum* may occur after 1 or 2 transfers, and the cultures used in the dye tests were transferred from 1 to several times between the tests, it seems reasonable to assume that variant forms may have been used in the later tests, which reacted differently to the toxic dyes than did the original cultures. Padwick (13) states, "A number of isolates of *F. udum* were compared with *F. lateritium* var. *uncinatum* Wr. and also with cultures of all varieties and physiologic forms of *F. vasinfectum*, obtained from the centraalbureau voor Schimmel cultures, Baarn. This experiment strikingly confirmed the previous conclusion that *F. udum* is a separate species from *F. vasinfectum*." The writers are not familiar with the 8 cultures of *F. vasinfectum* examined by Padwick, but one wonders what might have been the conclusion if the wide range of forms they have had in culture had been included in his study. Padwick was not able, however, to distinguish by morphological and cultural studies between the isolates of

F. udum that cause wilt only of pigeon-pea and those that cause wilt only of sun-hemp.

Snyder and Hansen (16) have obtained types of the watermelon wilt Fusarium that fall into sub-sections Orthocera, Oxysporum, and Constrictum of the section Elegans, as proposed by Wollenweber and Reinking (19). It was proposed by Snyder and Hansen (16) that the section Elegans be converted into 1 species, *F. oxysporum,* with a number of biologic forms recognized principally by their selective pathogenicities. According to this scheme, *F. vasinfectum* Atk. becomes *F. oxysporum* f. *vasinfectum* (Atk.) Snyder and Hansen, and *F. oxysporum* Schl. v. *nicotianae* Johns. becomes *F. oxysporum* f. *nicotianae* (Johns.) Snyder and Hansen.

When it was discovered that the Fusaria from Burley tobacco collected in South Carolina and Kentucky would also cause cotton wilt, and that the cotton wilt Fusarium would cause wilt of Burley tobacco (1), it was assumed that both names of the fungi were unnecessary. With the later discovery that the Maryland tobacco-wilt organism would not infect cotton and okra, it became clear that both names might be needed. The Maryland tobacco-wilt organism is also different from the other two tobacco organisms in that it causes wilt in the flue-cured variety, Gold Dollar, where the others do not. If one were comparing the several tobacco organisms by inoculating the susceptible Burley variety, the conclusion would be that they are the same. If the flue-cured variety were added to the tests, the different pathogenic potentialities could be indicated by designating them as different physiologic races. If cotton be added to the test, the situation becomes slightly more complex, since the Kentucky and South Carolina isolates also cause cotton wilt. On the basis of priority, the 2 latter isolates, according to the classification of Wollenweber and Reinking (19), should be *F. vasinfectum.* When increasing the host list of *F. vasinfectum* to include *Cassia tora* and tobacco, it would be necessary to state that this organism causes tobacco wilt in only certain types of tobacco (Burley strains). If the classification of Snyder and Hansen (16) be used, the name would be given as *F. oxysporum* f. *vasinfectum* with the increase in host list as suggested above. The Maryland isolate which affects Maryland Broadleaf and flue-cured types would retain the name of the tobacco organism as originally described.

Three series of cross inoculations involving the sweet potato have been made but no detailed data are presented at this time, since the results in general are rather uncertain. The South Carolina and Kentucky tobacco-wilt fungi have not produced distinct external symptoms of wilt in the sweet potato in all tests, though Smith and Shaw (15) have reported that the tobacco-wilt fungus with which they are working does cause wilting of the sweet potato.

Pathogenicity tests with the wilt-producing Fusaria are not short and easy procedures, but it appears that many cross-inoculation experiments are necessary before the pathogenic potentialities of this group will be known. The morphological and cultural studies thus far recorded fall far short of giving a satisfactory method for identifying many of these organisms.

SUMMARY

Inoculations with the wilt Fusaria from *Cassia tora*, cotton, tobacco, okra, tomato, watermelon, and cowpea have been made chiefly by means of a solution-culture technique, though some soil inoculations also were employed.

The isolates from cotton, okra, Cassia, and tobacco from South Carolina and Kentucky caused wilt with these various hosts; thus it appears that one fungus is involved. A susceptible Burley tobacco was attacked, but a flue-cured variety, Gold Dollar, showed little or no wilt when inoculated.

Tobacco isolates from Maryland, however, caused wilting of both the Burley and the flue-cured variety of tobacco, but not of cotton and okra.

The weed, *Cassia tora*, found growing in cotton fields was attacked by the cotton-wilt fungus. The tomato, watermelon, and cowpea fungi are probably pathogenic only to their respective hosts or closely related hosts, since the tomato fungus did not cause wilt of watermelon, okra, or cotton, the watermelon fungus did not cause wilt of tomato, and the cowpea fungus attacked neither cotton nor Cassia.

The following is a brief summary of the results of the inoculations.

Fusarium from	Caused wilt of	Caused no wilt of
Cotton	Cotton, Burley 5 tobacco, okra, Cassia	Gold Dollar flue-cured tobacco, watermelon
South Carolina tobacco	Cotton, Cassia, Burley 5 tobacco	Gold Dollar flue-cured tobacco
Kentucky tobacco	Cotton, Burley 5 tobacco	Gold Dollar flue-cured tobacco
Okra	Okra, Burley 5 tobacco, cotton	Tomato
Cassia tora	Cassia, Burley 5 tobacco, cotton	
Maryland tobacco	Burley 5 tobacco, Gold Dollar flue-cured tobacco	Cotton, okra
Tomato	Tomato	Watermelon, okra, cotton
Watermelon	Watermelon	Tomato
Cowpea	Cowpea	Cotton, Cassia

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THE EFFECT OF LIGHT AND TEMPERATURE ON THE VIABILITY OF UREDIOSPORES OF CERTAIN CEREAL RUSTS[1]

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INTRODUCTION

It has been shown that urediospores and aeciospores of some of the cereal rusts, notably *Puccinia graminis* Pers., may be carried long distances by the wind. According to Stakman (18), there is conclusive evidence that urediospores of *P. graminis* are sometimes blown northward from Texas, Oklahoma, or Kansas into the spring-wheat States in such numbers as to be responsible for the initiation of rust epidemics.

Urediospores of *Puccinia graminis* from a distant source often are caught near St. Paul, Minnesota, in large numbers on spore traps in June; and in July the numbers are sometimes almost inconceivably large. At this time, from a few hundred to a few hundred thousand urediospores may be deposited per square foot at the level of growing grain plants within 24 hours during so-called spore showers. These spores are blown into the spring-wheat area by south winds and very often during periods of rather warm, bright, dry weather, when there is insufficient moisture for them to germinate. It is well known that high temperatures and light are likely to affect adversely the development of the cereal rusts (1, 4, 7, 8, 9, 10, 12, 13, 14, 20, 22). These factors also affect the viability of spores, but to what degree has not been known very precisely.

As information regarding the number of spores caught in spore traps has been used as one basis for attempting to determine whether epidemics are likely to occur, it seemed desirable to get as much information as possible regarding the longevity of the spores on the plants with a view to establishing if possible a correction factor based on the amount of sunshine and the temperature and the time elapsing between the spore shower and the first dew or rain.

For this reason the writer attempted to determine the effect of sunlight and temperature on urediospores of *Puccinia graminis tritici* Eriks. and Henn., *P. coronata* Corda, *P. rubigo-vera tritici* (Eriks. and Henn.) Carl., *P. graminis avenae* Eriks. and Henn., and *P. graminis secalis* Eriks. and Henn. As races of a given species conceivably could differ in their resistance to light and high temperature, several physiologic races of *P. graminis*

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tritici and *P. graminis avenae,* as well as *P. coronata,* were included in many of the studies. There was no attempt to study relative humidity or any of the other factors that have been reported (5, 7, 15, 21) as reducing spore viability.

MATERIALS AND METHODS

Spores of *Puccinia coronata, P. graminis tritici,* and *P. rubigo-vera tritici* for the preliminary experiments were obtained from two different sources, the fields of University Farm, St. Paul, Minn., and plants grown in the greenhouse.

Rust collections used for the subsequent experiments were obtained from the Federal Rust Laboratory, University Farm, St. Paul, and propagated on seedlings in the greenhouse. The usual methods of inoculation and incubation were used (17) for races 11, 36, 38, and 56 of *P. graminis tritici,* races 2 and 6 of *P. graminis secalis,* races 1 and 45 of *P. coronata,* and *P. rubigo-vera tritici.* As soon as uredia were well formed, mature spores were collected by gently shaking the infected plants over a smooth funnel leading to a glass vial. The conditions for spore production among different rusts were almost identical, unless specific treatments were given for certain purposes.

Methods of Exposure

Immediately after the spores were collected from the greenhouse, they were put on a piece of white paper over a piece of filter paper in Petri dishes. The dry spores were evenly distributed before exposure to the direct sunlight, because Fulton and Coblentz (3) pointed out that the outer spores of clumps have a protective shading effect on those in the center of the mass, and the latter survive exposures several times longer than is required to kill single detached spores. Usually, the dishes were covered with white cellophane and checks were covered with black tar paper, although no covers were used in the preliminary experiments. When testing the varying qualities of daylight, blue cellophane and red Du Pont cellophane, with known percentages of light transmission, were used as covers.

These Petri dishes of dry spores were exposed continuously outdoors; but, after a certain specified number of hours, they were carried back to the laboratory and a small amount of each sample was removed with sterile forceps and placed in separate, clean Syracuse dishes. The Petri dishes were again put outdoors for further exposure and later samplings.

The records for temperature and light intensity were usually taken 3 times a day. Thermometers were inserted in Petri dishes covered as for spore exposures. The instrument used to measure light intensity in the preliminary experiments consisted of a glass bulb fitted with a thermopile, and the record was in terms of g. cal./min./cm.^{2} For the succeeding experiments, a Weston Illumination meter, model 603, was used and light intensity was recorded in foot-candles. According to Duggar (2) Kimball gave an average value of 6700 foot-candles for 1 g. cal./min./cm.^{2} The former records, therefore, were converted into foot-candles based on Kimball's method.

The initial germination in a spore collection varied from time to time and also with the different rust species used. Usually it was between 70 and 100 per cent, but occasionally for certain spore collections, it fell to 50 or even lower. In all cases in order to have the rates of decline in viability more comparable, the initial germination percentage for every spore lot was considered as 100. Subsequent germination of each spore lot after exposure was figured as a proportion of this base.

Methods for Spore Germination

After a certain number of hours' exposure, spores were distributed in Syracuse dishes for germination tests. Distilled water was added to the dishes, which were then left for 3 to 10 hours at room temperature (20–25° C.) before spores were counted. Temperatures were relatively uniform, for when spores of different races of certain species of rusts were tested for germination they were always kept side by side. Approximately 10 cc. of water, distilled in a metal still, were used for each Syracuse dish. The total number of spores counted in each dish was from 300 to 500. Usually, at least 3 tests were made for each species, variety, or race of rust in each experiment, but only representative data are presented here.

THE GERMINATION OF UREDIOSPORES FROM DIFFERENT SOURCES

Urediospores produced in the greenhouse were compared with those from the field as to their ability to withstand exposure to air and light, for it was realized that the source of spores, especially during the winter, might be an important factor in experiments on viability. Mature urediospores of *Puccinia graminis tritici, P. coronata,* and *P. rubigo-vera tritici* from the two sources (greenhouse and field) were exposed continuously outdoors during July, 1937, and their germinability tested after intervals varying from 2 to 125 hours. There was no evidence of marked differences in viability of the urediospores from the two different sources, so that for the later experiments urediospores produced in the greenhouse were used. This practice enabled one to use pure races of rusts, to have material available in all months of the year, and to control to a slight extent the conditions for urediospore production.

COVERING MATERIALS FOR PETRI DISHES AND THEIR EFFECT ON LOSS OF UREDIOSPORE VIABILITY

Since exposure in uncovered dishes was impractical for the outdoor experiments whenever large numbers of spores were in the air, when strong winds or storms prevailed, or when snow or rain fell, various covering materials were used, including white cellophane, glass, a double layer of cheesecloth, and black tar paper. The cellophane interfered very little with passage of light waves, but, in windy or stormy weather, it split easily.

Cheesecloth reduced light intensity only slightly, but did not prevent disturbance and loss of the exposed spores. The black paper reduced the light greatly and the temperatures under such covers were apt to be higher than those under glass or cellophane covers. The results of numerous experiments, however, show clearly that, provided temperatures are not extremely high, viability of urediospores is retained much longer under a black cover that reduces light than under a cover that transmits most of the light reaching it. In table 1 are given figures for the germinability of urediospores of race 11 of *Puccinia graminis tritici,* which may be taken as representative of the other rust races and varieties studied. *P. rubigo-vera tritici, P. coronata,* and races 36, 38, and 56 of *P. graminis tritici* were included in the experiments, and the results for all were very similar in each experiment.

Obviously, there were physical difficulties in starting all the exposures, the replicates, and all the different race collections at the same time, but care was exercised in observing, according to the starting sequences. Insofar as possible, exposures were begun between 7:30 and 9:00 A.M. Length of exposure is recorded in hours, but, since sunlight was the important factor being investigated, the number of hours of sunlight during exposure usually has been given as well. Petri plates exposed more than 8 hours were left out continuously day and night, and the total time of exposure was 2, 3, or more times greater than the hours of daylight recorded.

With exposure to daylight under a cellophane, a glass, or cheesecloth cover viability decreased rapidly at low and moderate temperatures, more rapidly than with the black paper cover (Table 1). As long as temperatures remained moderate, such protection from light by the black tar paper cover provided for retention of viability for 4 to 8 days or even 24 to 28 days longer than did a transparent cover.

TABLE 1.—*The percentage germination of urediospores of Puccinia graminis tritici race 11 after exposure, under different covers, to daylight and to various temperatures*

Exposure in hours[a]	− 10° to + 6° C.			12° to 26° C.				27° to 42° C.	
	Low light		Black paper	Low light		Black paper	High light	High light	Black paper
	Cloth	Glass		Cloth	Cellophane		Cellophane	Cellophane	
0	100	100	100	100	100	100	100	100	100
8 (8)	2	2	24	61	41	69	55	20	tr
15 (8)	....	....	....	....	....	....	29	3	0
30 (15)	1	2	6	16	14	27	1	0	0
50 (20)	3	1	5	11	12	18	tr	....	....
100 (40)	1	1	5	14	7	10	....	....	....
150 (60)	0	0	4	6	7	5	....	....	....
400 (153)	....	....	5	1	1	4	....	....	....
460 (175)	....	....	5	0	tr	1	....	....	....
500 (190)	....	....	5	0	0	1	....	....	....
600 (225)	....	....	2	....	....	tr	....	....	....
800 (300)	....	....	1	....	....	0	....	....	....
1000 (400)	....	....	tr	....	....	....	....	....	....

[a] The number in parentheses indicates the number of hours of sunlight during the exposure period.

Whenever temperatures were high, then temperature assumed a primary role and daylight became an accessory factor in loss of urediospore viability (Table 1). In nature, however, it is only occasionally that temperature rises so high as to become a primary factor for urediospore viability of the rusts considered in this paper. In nature it is possible that the cereal foliage reduces sunlight intensity, and temperature as well, and prolongs somewhat the germinability of urediospores.

THE EFFECT OF HIGH TEMPERATURES ON THE VIABILITY OF UREDIOSPORES OF SEVERAL CEREAL RUSTS

Since there was evidence that spores were killed by high temperature, as well as by light, the germination of spores kept in incubators at 44 ±, 50 ±, and 60 ± ° C., respectively, was studied. Three experiments with 3 replications for each rust were made at different dates. Mature spores of the 4 races of *Puccinia graminis tritici*, the 2 of *P. graminis avenae, P. graminis secalis, P. rubigo-vera tritici*, and the 2 races of *P. coronata* were exposed.

Data for a representative sample, race 36 of *Puccinia graminis tritici*, are presented in table 2. Urediospores withstood a temperature of 44° C. very well for 2 days; and, even after 60 hours, 8 per cent of the spores germinated.

At 50° C. viability was lost more rapidly, so that, after 2 days, only 1 per cent of the spores germinated, and within 60 hours all were dead.

Exposure to 60° C. brings about a still more rapid decline in germinability. Within 4 hours, more than half the spores lost their viability, and at the end of 10 hours less than 10 per cent were able to germinate. Nearly all spores were dead after 15 hours at 60° C.

TABLE 2.—*The effect of high temperature on the viability of urediospores of Puccinia graminis tritici 36*

Exposure in hours	Average percentage[a] germination of spores after exposure at		
	44° C.	50° C.	60° C.
0	100	100	100
8	95	45	26
10	75	41	8
15	79	23	tr
20	67	11	0
25	52	12	0
36	40	4	0
48	28	1	0
60	8	0	0

[a] Each figure is the average of three replicates.

Attempts were made to harden certain spore lots by exposure for seven hours at room temperature before the exposure to 60° C. Such treatment, however, did not enable the spores to withstand the high temperature any better than did nontreated spores.

In nature we may safely say that such high temperatures never obtain in the Upper Mississippi Valley, although 40° C. and slightly higher have been recorded on occasion in Minnesota. Intense sunlight in nature, however, undoubtedly adds its inimical effects to those of high temperature, and the viability of urediospores in the air is reduced more rapidly than when either factor acts alone.

LIGHT INTENSITY AND LOSS OF UREDIOSPORE VIABILITY

It is difficult to separate the factors of light intensity and temperature when studying retention of viability in rust urediospores, but two series of experiments are available in which the predominant range of temperature was 14 to 24° C. in one instance and 16 to 24° C. in the other, while the light intensity was held to a low limit in one but soared to 7,000 foot-candles in the other. Data for the germinability of races 36, 38, and 56 of *Puccinia graminis tritici* under these two sets of conditions are in table 3.

One criticism of the work might be that the experiments occurred in two different years. Nevertheless both lots were collected from the greenhouse at about the same time of year (within 7 weeks), and the initial germination percentages were very similar in both series. With the practice of setting the initial germination count at 100 per cent, the comparison should be a very good one.

A direct correlation between the lower light intensity and retention of viability is evident. During the first 3 days of Experiment II the average light intensity was rather low, and it was not until the fourth day that light intensity increased enough to differentiate the data of Experiment II from those of Experiment I.

Germination counts for *Puccinia graminis tritici* race 11, for *P. coronata*

TABLE 3.—*The effect of light intensity on loss of viability, as measured by percentage germination, in urediospores of races 36, 38, and 56 of Puccinia graminis tritici*

Exposure[a] in hours	500–1500 foot-candles			1000–7000 foot-candles		
	Race 36	Race 38	Race 56	Race 36	Race 38	Race 56
0	100	100	100	100	100	100
8 (8)	87	82	78	96	100	85
25 (10)	....	....	....	70	78	69
50 (20)	....	....	....	58	72	72
80 (36)	18	50	39	58	15	38
123 (48)	16	40	34	42	26	41
150 (60)	....	....	....	27	20	26
175 (75)	15	37	33	13	10	28
225 (100)	14	28	33	....	....	....
300 (140)	9	24	29	0	0	tr
365 (175)	8	25	30	....	....	....
420 (200)	3	21	28	0	0	0
570 (270)	1	15	14	....	....	....
650 (300)	1	2	2	....	....	....
750 (350)	0	1	tr	....	....	....
840 (430)	0	tr	tr	....	....	....

[a] The number in parentheses indicates the number of hours of sunlight during the exposure period. The temperature ranged from 14 to 24° C.

races 1 and 45, *P. graminis avenae* races 2 and 6, and *P. graminis secalis* are very similar to those presented and would have illustrated the effect of light intensity as well as the data chosen for table 3.

At low light intensities more than 10 per cent of the spores remained viable after exposure to sunlight for 270 hours, except for race 36 of *Puccinia graminis tritici* whose viability dropped more rapidly. The 270 hours of sunlight at the time of the experiments in February and March means about 23 to 24 days of exposure, a relatively long period for urediospore survival in the air.

When light intensity reached a daily maximum of 7,000 foot-candles, at least 10 per cent of the urediospores retained the power to germinate after a 75-hour exposure, but none survived 270 hours of sunlight. With higher light intensities, which sometimes surpass 10,000 foot-candles on a June day in Minnesota, the survival time for urediospores may be even shorter.

QUALITY OF LIGHT AS IT AFFECTS UREDIOSPORE VIABILITY

The short waves of the ultra-violet and the blue parts of the spectrum generally have been more effective in decreasing germinability of fungus spores than have the longer waves (2, 3).

Cellophane Filters

In order to determine the relative effects of different qualities of direct sunlight, filters of 3 colors of Du Pont cellophane, with known percentages of transmission, were used to cover the exposure dishes. The colorless, red, and blue filters were compared with black paper covers for their effects on germinability of urediospores of all the rust species, varieties, and races used in other experiments.

All spores for these experiments were collected during October through December in 1938 or in 1939, and the initial germination counts for all spore lots were usually rather low. As in all other experiments, the germination counts after various exposure periods represent percentages of the initial germination counts before exposure.

From table 4 it is evident that urediospore viability declines more rapidly under the colorless cellophane filter than under the blue and red cellophanes or the black paper covers. The data presented for race 2 of *Puccinia graminis avenae* are representative of the other races, varieties, and species of rusts used in 8 different series of experiments, except *P. graminis secalis*. For this variety the loss of viability was about equal under all covers, and it is possible that its spores are slightly more resistant to sunlight than those of other varieties and species.

Ultra-violet Light

In October and November, 1938, mature urediospores were irradiated with ultra-violet, and their germination tested after various intervals. The spores were evenly distributed in Syracuse dishes within Petri dishes, which were left open for irradiation or were covered with colorless cellophane. A slanted board, 32 inches from the light source, supported the dishes for irradiation. The Eveready Therapeutic "C" carbon arc furnished wave lengths of 2,700–3,000 Ångstrom units (see 11), and the temperature at the irradiation distance was between 30 and 36° C., while measurable light intensity was about 800 foot-candles. Spores were irradiated 15 or 20 minutes, then tested for germination in distilled water at room temperature.

TABLE 4.—*The percentage germination of urediospores of Puccinia graminis avenae race 2 exposed under covers of colored cellophane to light of different quality*

Exposure[a] in hours	Colorless	Blue	Red	Black
0	100	100	100	100
8 (8)	48	75	75	59
25 (10)	23	15	15	16
32 (15)	7	7	10	8
54 (20)	3	3	10	3
98 (35)	2	10	14	13
144 (48)	0	9	13	13
215 (72)	0	3	9	9
290 (96)	0	1	5	4

[a] The number in parentheses indicates the number of hours of sunlight during the exposure period. Sunlight intensity was approximately 3500 to 6000 foot-candles and temperature was 12–27° C.

There is a general tendency toward reduction in germination after exposure for 15 minutes to ultra-violet light (Table 5), yet a surprisingly large number of spores were not killed by the 20-minute exposure.

Urediospores of *Puccinia graminis avenae* seemed to be more sensitive to ultra-violet than those of the *secalis* and *tritici* varieties of *P. graminis* or those of other rust species. Direct irradiation was often slightly more injurious than irradiation through cellophane, but it was not consistently so.

In each case one series of figures was obtained for spores collected and stored for a week at 10° C. before exposure to ultra-violet and another series of figures for spores collected and exposed immediately. Storage of spores sometimes seemed to confer a very slight resistance to ultra-violet, but the effects were inconsistent.

CONDITIONS OF SPORE PRODUCTION THAT MIGHT AFFECT UREDIOSPORE VIABILITY

Time of Formation in the Uredium

A single uredium in nature produces urediospores continuously for many days or sometimes even for weeks before the uredium changes to a telium or before it dries and stops drawing nutriment from the host. Stakman and Levine (19) showed that there was no apparent difference in the degree of infection produced by rust spores of different ages, but it was not known whether urediospores, formed early in the life of the uredium, would be more or less resistant to the effect of sunlight than those formed late in the life of the uredium, when supplies of the host nutriment might be more or less depleted.

Early crops of mature spores were collected soon after uredia had formed, usually 12 or 13 days after inoculation. Seven or 8 days later another crop of mature spores was collected from the same uredia. In some of the experiments, by storing the first spore collections at 10° C. for a week, it was possible to expose both collections to the same light and temperature and compare their relative sensitivities. According to the reports of Hoerner (6), Peltier (13), Schilcher (16), and Rosen and Weetman (15), short storage does not reduce the vitality of spores to any considerable extent.

TABLE 5.—*The percentage germination*[a] *of urediospores of various species of Puccinia irradiated with ultra-violet light directly or through cellophane covers*

Rust urediospores	Irradiation, 15 min.		Irradiation, 20 min.	
	Cover	No cover	Cover	No cover
P. gr. avenae 2	22	18	13	15
do 6	7	7	7	6
P. gr. secalis	80	76	65	69
P. gr. tritici 11	82	75	75	72
do 36	62	60	58	52
do 38	54	41	50	43
do 56	57	55	40	33
P. coronata 1	85	78	81	75
do 45	78	66	52	49
P. rubigo-vera tritici	75	65	71	64

[a] The percentage germination without irradiation was arbitrarily placed at 100.

In none of the experiments were there evident any consistent differences in sensitivity to light in the two spore crops. Loss of viability proceeded at the same rate in both collections when the exposures to light were comparable. When the two spore crops were subjected to 50° or 60° C., the spores of the later crop sometimes withstood extreme temperatures for a few hours longer than spores of the early crop. At moderate temperatures, however, no differences were evident. Apparently the spores formed early in the uredium mature rapidly and are as vigorous as those formed a week later, when the uredium usually is considered in its prime. Whether still later crops of urediospores would have a lower vitality or a greater resistance to high temperatures and drying is still a question.

Light Condition During Uredial Development

Throughout the years during which the present work was done, there were general observations on the relative initial vigor of spore lots collected at different seasons or under different environmental conditions. Indications are that urediospores formed during long-day seasons, when sunshine is prevalent and relatively bright, are more vigorous as measured by a higher initial germinability than are urediospores formed during a short-day winter season, when cloudy days prevail. With nearly all of the rusts studied here, urediospores formed in the greenhouse during November or December germinated in fewer numbers (usually from 35 to 70 per cent) when freshly collected than did urediospores formed in any other month of the year (usually 75 to 95 per cent). The single possible exception seemed to be *Puccinia graminis secalis*, a variety in which initial germination of urediospores formed in November or December sometimes was remarkably high (86 per cent).

To determine whether a greater than usual reduction in light during November and December would result in more pronounced weakening of urediospores formed in those months, half-shade was provided for inoculated plants by 1 or 2 layers of muslin. Urediospores produced under half-shade were compared with those produced under the full light of November and December, and there were no consistent differences in initial germinability of the freshly collected spores or in the decline in germinability resulting from exposure to sunlight or to high temperatures.

THE EXPOSURE OF RUST SPORES ON CEREAL FOLIAGE AND THE INFECTION OF PLANTS AFTER EXPOSURE

In the normal course of a growing season many of the urediospores, drifting or being blown about by air currents, eventually settle on grain fields and cereal foliage. Environmental conditions are not always favorable for immediate germination and infection, so the deposited spores often are exposed to the elements for several days longer. As yet, no one is certain whether most of the spores retain their ability to infect the cereal host for some time after deposition or whether only a few are able to do so. It is possible that under field conditions the foliage protects urediospores from the full effects of direct sunlight and that it modifies temperature so that many spores survive. The experiments concerning this part of the work were made with seedlings growing in 4-inch pots in the greenhouse. The susceptible wheat variety Marquis and the moderately resistant oat variety Anthony were brushed with dry urediospores of their respective rusts about 5 or 6 days after planting, when the seedlings were 2 to 3½ inches high. Immediately after the spores were brushed on, some of the plants were exposed to direct daylight in the greenhouse, others were exposed to diffuse daylight while in a shaded place in the greenhouse, and a few were put in moist chambers and atomized lightly with water to allow for immediate infection and to serve as checks for the exposed plants. After exposure for a designated length of time the seedlings were placed in a moist chamber, atomized lightly with water, and left for 48 hours.

The success of infection was determined by counting the lesions that developed on plants within 10–12 days after the moist chamber incubation

(Table 6). The number of uredia on a plant was somewhat erratic, which is not surprising when one realizes how difficult it is to brush spores evenly over the leaf surfaces and to avoid washing off spore clumps during the atomizing. Nevertheless, uredia were numerous enough to signify that considerable numbers of spores remained viable and infected the cereals when opportunity arose. Even after exposures of more than 4 days, with about 48 hours of direct light, good rust infection resulted within 10 or 12 days after moisture was supplied for spore germination. With the longer exposures, particularly on wheat, a diffuse light was less harmful to spore viability than was direct light. High temperatures at the beginning of the experiment may have been responsible for the rather low averages for the check plants in the experiments.

We may conclude, however, that many of the urediospores deposited on cereal leaves, especially on the lower shaded foliage that is protected from direct sunlight, may remain viable for at least 4 days and probably longer, there being no necessity for moisture in amounts that would allow for rust infection at the exact time of the spore shower.

TABLE 6.—*The average number of uredia formed on oats and wheat seedlings that were dusted with urediospores of Puccinia coronata, P. graminis tritici 36, of P. rubigo-vera tritici and were exposed to sunlight for various times prior to incubation in moist chambers*

Exposure[a] in hours	*P. coronata*		*P. gr. tritici* 36		*P. rubigo-vera tritici*	
	Full light	Diffuse light	Full light	Diffuse light	Full light	Diffuse light
0	20	20	10	10	18	18
8 (8)	34	29	34	26	61	68
10 (10)	32	28	29	10	40	66
28 (15)	23	22	36	35	48	71
46 (20)	29	41	18	17	25	52
51 (25)	13	27	17	15	5	55
75 (36)	4	2	10	19	3	27
100 (48)	2	6	4	20	2	36

[a] The number in parentheses indicates the number of hours of sunlight during the exposure period. Light intensity was approximately 5000–7500 foot-candles, and temperature was 18–27° C.

DISCUSSION AND CONCLUSIONS

It has been known for some time that urediospores of the cereal rusts can be carried long distances by the wind and that many of the spores are viable after several days in the air. They often survive fairly long journeys in the air and serve as inoculum for cereal fields at some distance from their source. Among the factors that are unfavorable for urediospore survival are high light intensity and high temperature, which are discussed in this paper. It has been shown that urediospores are sensitive to exposure to sunlight, and the present work demonstrates a direct relationship between light intensity and the rapidity with which viability is lost.

On the basis of the present experiments, it is probable that at low light intensity some of the urediospores in the air remain viable for more than a month, but at moderate light intensities, with a daily maximum of 7000 foot-candles, which may be expected in May and early June in Minnesota, most of the spores in the air lose their viability in 12 to 14 days. With greater light intensities it is estimated that the spores survive an even shorter time.

In spite of the seemingly rapid loss of viability by most of the spores when light intensity is high, there would still seem to be a fair margin of safety for spore survival and for effectiveness of inoculum in an ordinary season. The heavy spore showers occur when days are long and bright, but the number of spores is generally so high that a small percentage of viable spores is sufficient to produce good rust infection. Furthermore, spores may be carried at a rapid rate by the prevailing winds, so that 1 to 3 days suffice for transfer of inoculum to new, far-distant, susceptible fields. Furthermore, although bright sunny days are expected in June, there usually are 1 or 2 cloudy days, or days of diffuse light, alternating with 3 to 5 sunny days. It is not until July that we expect long stretches of days with bright sunshine and high temperatures throughout the Upper Mississippi Valley.

High temperatures as well as intense light are detrimental to survival of urediospores. In nature, however, it is doubtful if temperatures ever rise to such heights as were used in the experiments, and if they do occur during the growing season for cereals, seldom last very long.

Survival of urediospores after they are deposited on cereal foliage is another of the important aspects of the rust epidemiology situation. Although urediospores may survive several days in the air, several additional days may elapse after spores are deposited on cereal plants before sufficient moisture becomes available for spore germination and infection. It appears from the present study that deposited spores may be somewhat protected from the adverse effects of light and temperature and that many of them may survive on the dry foliage surfaces for longer than 4 days.

It may be assumed, therefore, that in most seasons large percentages of the urediospores present in the atmosphere at a given time may lose their viability before there is opportunity for them to function as inoculum for new cereal plantings, but that relatively small proportions of the vast numbers of urediospores serve to spread and perpetuate the rust.

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INDEXING CHERRY YELLOWS ON PEACH

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INTRODUCTION

In 1939, Keitt and Clayton (5) reported a bud transmissible chlorosis on Montmorency cherry (*Prunus cerasus* L.) in Wisconsin. This disease, characterized by yellow leaves and premature defoliation, probably had been present in the commercial districts of Wisconsin, Michigan, and New York (8) for at least 20 years prior to this discovery. The delay in identification is understandable when the various agents in the leaf-yellowing complex in sour cherries are considered—sprays, insects, drought, fertilization, fungi, mechanical injuries, and a virus.

Cherry yellows spreads relatively slowly in orchards, probably through the instrumentality of a yet unknown insect vector. Moreover, it is not an aggressive disease (4, 6, 7) and does not kill the trees, but gradually reduces fruiting to a commercially unprofitable level. The yellowing and dropping of the older leaves in early summer each year causes a decline in tree vigor in which the spur system is markedly reduced until fruit bearing becomes confined principally to the previous season's growth (Fig. 1, A). Since the fruit is often larger than normal, and of excellent quality, growers commonly retain such trees, even after they become unprofitable producers.

Cherry yellows has been found in nursery plantings (3). In the nursery, bud sticks for propagation purposes are ordinarily taken from the nursery and budded from row to row in succession year after year. Occasionally, however, bud sticks are collected in orchards. In any case, propagation of cherries takes place in late summer after the most conspicuous yellow-leaf drop stage has passed. Owing to these circumstances it is quite apparent how the yellows disease can get into nursery trees through the unwitting use of diseased scions. Besides there is the possibility of entry through the root stock.

The New York control program for cherry yellows has thus far been confined to the elimination of the disease from the nurseries (3). With this end in view a two-fold program was undertaken in 1941 involving: (1) the eradication of diseased plants already present in nursery plantings, requiring the proper training and supervision of the horticultural inspectors and (2) the selection of disease-free propagation materials, requiring the indexing and certifying of both scion variety and understock. Consequently, the development of a quick and reliable indexing technique for demonstrating the presence of the yellows virus in cherry was considered of paramount importance in the research program.

PRELIMINARY EXPERIMENTS AND OBSERVATIONS

It has been demonstrated experimentally and also observed in a nursery planting that cherry yellows propagates readily and produces symptoms in the first year's growth from the bud, that is, with the production of symptoms in about a year's time from budding. This corresponds to the rate of transmission in orchard trees (5, 6, 7). While the details are being omitted for the sake of brevity, certain points deserve mention. On young nursery stock disease symptoms have a tendency to appear somewhat later in the season than on orchard trees, perhaps because nursery plants continue to grow later. Also on individual nursery plants symptoms may take the expression of many diseased leaves or be as inconspicuous as one chlorotic spot on a single leaf. In some cases first-year cherry grafts may first show symptoms as late as September; in others, trees presumably carrying the virus may show no symptoms the first year.

Similarly it has been demonstrated both in the greenhouse and in an experimental planting that diseased cherry buds propagate the disease in seedlings and in at least one grafted variety of peaches. On the peach the symptoms ordinarily appear the year following budding, and the expression consists primarily of a stunting in internodal growth, which crowds the leaves into clusters simulating rosettes. Following budding, areas of dead bark may appear in close proximity to the buds, and by the time symptoms are showing the following season the shoots receiving the diseased buds may be entirely dead, presumably from the yellows virus. However, a similar necrotic condition, along with rosette symptoms, has been frequently induced on peach with buds from a ring-spot disease,[1] a common contaminant in yellows-infected cherry orchards.

Besides speeding up symptom expression, it was noted that when the inoculated shoots were pruned back to the neighborhood of the diseased bud, necrosis was intensified with the dieback condition extending down the stub several inches within a month from pruning. Manifestly, much more work is needed on this phase of the study before drawing conclusions.

The discovery that the incubation period of yellow-red virosis on peach could be greatly reduced by a simple pruning technique (1) suggested testing this rapid transmission technique on cherry yellows (2).

1941 Transmission Experiments

Materials and Methods. Several test plants were used in this study. The so-called second-year Elberta peach trees were budded in the nursery and grown 1 year before being dug. The yearling peach seedlings had grown from seed one season in the nursery. After digging in late autumn they were potted and placed out doors for 2 months before forcing in the greenhouse. The yearling peach grafts were part of the same lot of seedlings that were budded to Elberta in early September.

The small peach seedlings were started from seed (southern, natural) and ready for germination after storage for 10 weeks in moist peat at 5° C. After germination in sand, the seedlings were potted in soil and reached the desired height (about 18 inches) in approximately 18 weeks from the start of the cold treatment. Lots of several hundred pits each were started at monthly intervals so as to provide a supply of seedlings throughout the year. The seedlings were selected for uniformity and kept free from branches on the lower two-thirds of the stem prior to being used.

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[1] Besides ring spot, the sour-cherry-virus complex probably involves still other diseases about which too little is known at present for reliable identification.

Two severe insect pests—red spider and broad mite—encountered in the greenhouse were kept in check by a routine spray application of NNOR 1–400 at weekly intervals during the summer months. No spray injury resulted from repeated use of this material when applied early in the day.

The virus was obtained principally from diseased Montmorency cherry trees in Western New York, although inoculum for one of the transmission experiments also came from 3 other susceptible varieties. Depending on the season, it was taken from dormant and actively growing trees in nursery and orchard as indicated in the several experiments. The so-called "diseased sources" usually consisted of bud sticks obtained from separate trees in one or more orchards or nurseries. The dormant bud sticks were soaked in water for 2 days before use.

The ordinary inoculation technique consisted in inserting cherry buds about midway up the stem of the growing peach seedling and wrapping with "sterilastic" bandage. For rapid transmissions (2) the stem was cut off 1 node above the diseased bud from 0 to 7 days after budding.

Results of Transmission Experiments

Experiment 1. The first bud-transmission test from cherry to peach resulted in the production of ring-spot symptoms within 2 weeks from budding. It employed second-year Elberta peach grafts and a variation of the rapid transmission technique, especially useful with older plants, which consisted of placing the dormant diseased cherry bud on one side of the stem between 2 rapidly growing young shoots. The plants had previously been pruned to about 12 inches above soil level when brought into the greenhouse and, after shoot growth started, all but the desired shoots were removed and the plants budded. Budwood from 3 different sources were each tested on 2 peach seedlings on March 6. Four unbudded plants were kept as checks. Ten days later faint chlorotic rings were beginning to appear on the leaves of one shoot underneath the diseased bud on one plant and by the 14th day became more definite. At the end of 20 days many of the chlorotic rings had become necrotic and symptoms were showing also on the shoot above the diseased bud (Fig. 1, D). Only 2 of the 6 original plants showed these symptoms, and, as time passed, the chlorosis had not spread to the newer formed leaves and, although there was, in comparison to the checks, a marked stunting of growth on all 6 plants, stunting alone was not considered a positive symptom.

Experiment 2. An exploratory experiment was next conducted that resulted in the transmission of cherry-yellows virus from cherry to peach with the production of rosette symptoms within a month from budding. It employed yearling peach seedlings as test plants and Montmorency cherry

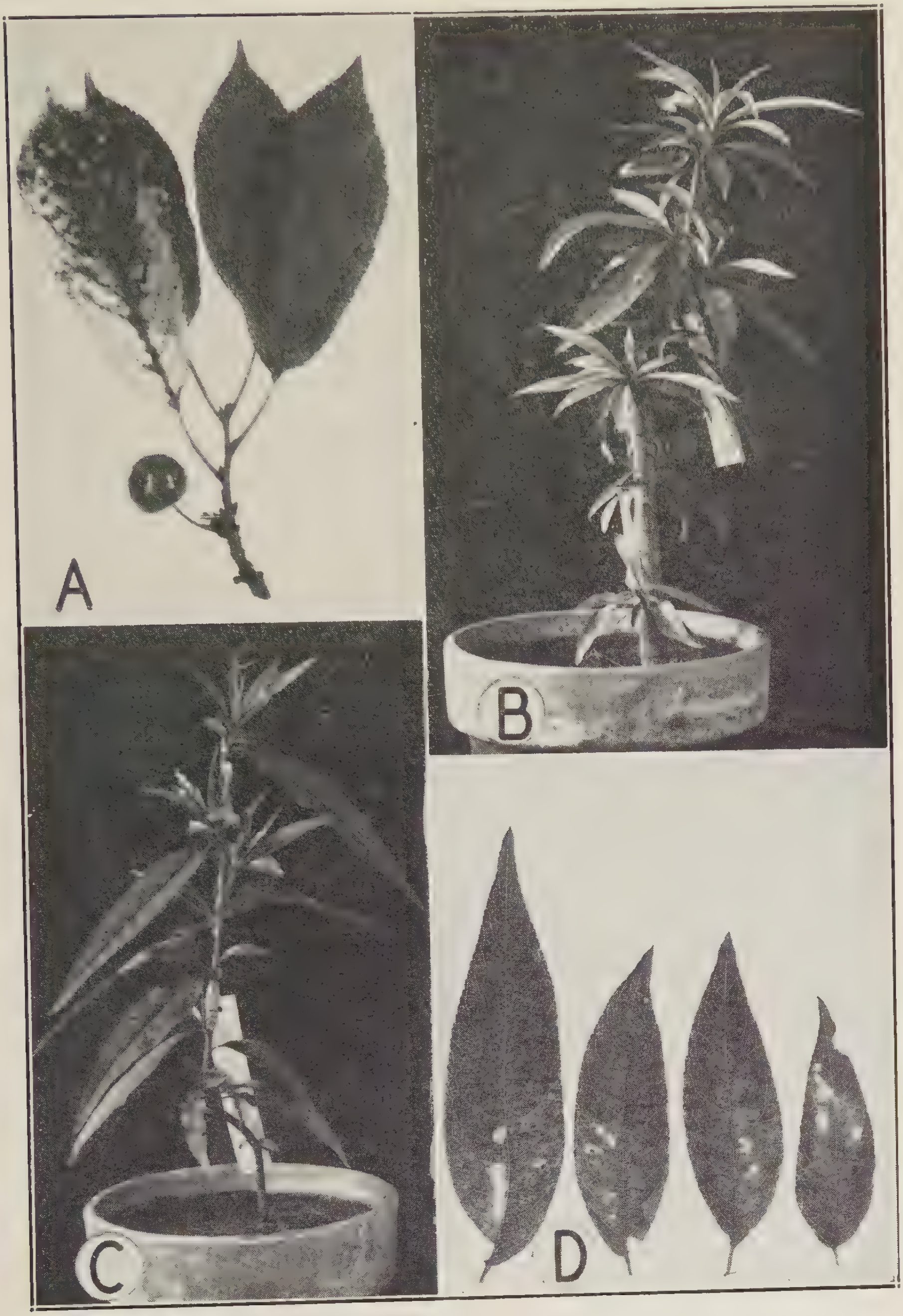

FIG. 1. A. Characteristic shoot condition late in July on a Montmorency cherry tree severely affected with yellows. Four yellow leaves were removed by the main wave of defoliation several weeks previously. B. Characteristic stunting or rosette symptoms on seedling peach 11 weeks after budding from Montmorency cherry affected with yellows. This condition persisted throughout the season. C. Typical early stage symptoms of chlorotic ring spot and rosette induced on seedling peach three weeks after budding from cherry. This plant was pruned on the day of budding. D. Chlorotic followed by necrotic ringspot symptoms induced on Elberta peach by bud inoculation from Montmorency cherry affected with yellows.

budwood from 1 healthy and 16 diseased sources. Of the diseased samples, 11 were from dormant trees collected in New York orchards, 4 from growing 2-year-old cherry trees previously inoculated by grafting from 1 New York and 3 out-of-State sources, and 1 from a cherry yellows infected peach seedling in the previous experiment.

On April 23, 3 seedlings were budded from each of 16 diseased trees from 8 different orchard sources, and 8 from a healthy nursery source. The point of bud insertion was about 12 inches above soil level on either the main stem or a side branch. Seven days later 2 of the 3 trees receiving the diseased buds and all 8 receiving healthy buds were cut back to 1 node above the inserted bud to induce new growth. For purposes of comparison, 9 yearling Elberta grafts received diseased buds from 1 source and at pruning time 3 were left unpruned.

Definite symptoms were first observed on 10 of the inoculated plants 18 days after pruning or 25 days from budding. The new spurt of growth stimulated by pruning showed occasional leaves with chlorotic rings, some of which became necrotic and later fell out, leaving perforations. At this stage the internodal growth was already definitely shorter and the leaves smaller in the inoculated plants than in the checks. Subsequently, the chlorosis disappeared while the stunting of growth persisted. A rather marked rosette condition, which persisted throughout the season, is shown in figure 1, B. This plant was photographed on July 15 or slightly under 3 months from budding. The stunted rosette condition was not always so marked as in this illustration nor the leaves so chlorotic. In some cases chlorosis was totally absent and rosetting still very pronounced.

Positive transmission, based primarily on the stunted rosette symptom, was obtained in 1 or more plants in 15 out of the 16 diseased sources tested. Three months from the start of the experiment symptoms were evident in no case in the unpruned inoculated trees or in the checks receiving buds from the healthy cherry sources. The uninoculated check trees all grew normally.

Similar but less striking results were obtained with the Elberta peach grafts. However, only 2 of the 6 inoculated trees showed positive symptoms. Since more of these plants were not available, the phase of the work with grafted varieties was discontinued.

Of interest was the observation that both of the peach seedlings receiving diseased peach buds became diseased. This indicates that cherry-yellows virus can be transmitted from peach to peach.

Experiment 3. The third bud-transmission experiment, which employed young 18-inch peach seedlings and the pruning technique, resulted in the production of positive symptoms in 23 days from budding. This experiment, which involved the indexing on peach seedlings of diseased cherry material from 4 sour-cherry varieties, gave more striking results than were obtained on the older seedlings in previous experiments. The cherry material used in this experiment was collected from 2 nurseries and 1 orchard in July, and consisted of 7 diseased, 2 doubtful, and 1 healthy sources. Of the diseased-bud sources 4 were Montmorency, and 1 each of Early Richmond, Chase, and English Morello. The peach seedlings were started from seed and budded when they were large enough to receive cherry buds about midway on the stem or when about 18 inches tall. Two trees received buds from each source. Four check trees received buds from the healthy source. Bud inoculations were made on July 25, and the tops were pruned back to 1 node above the diseased bud on the following day.

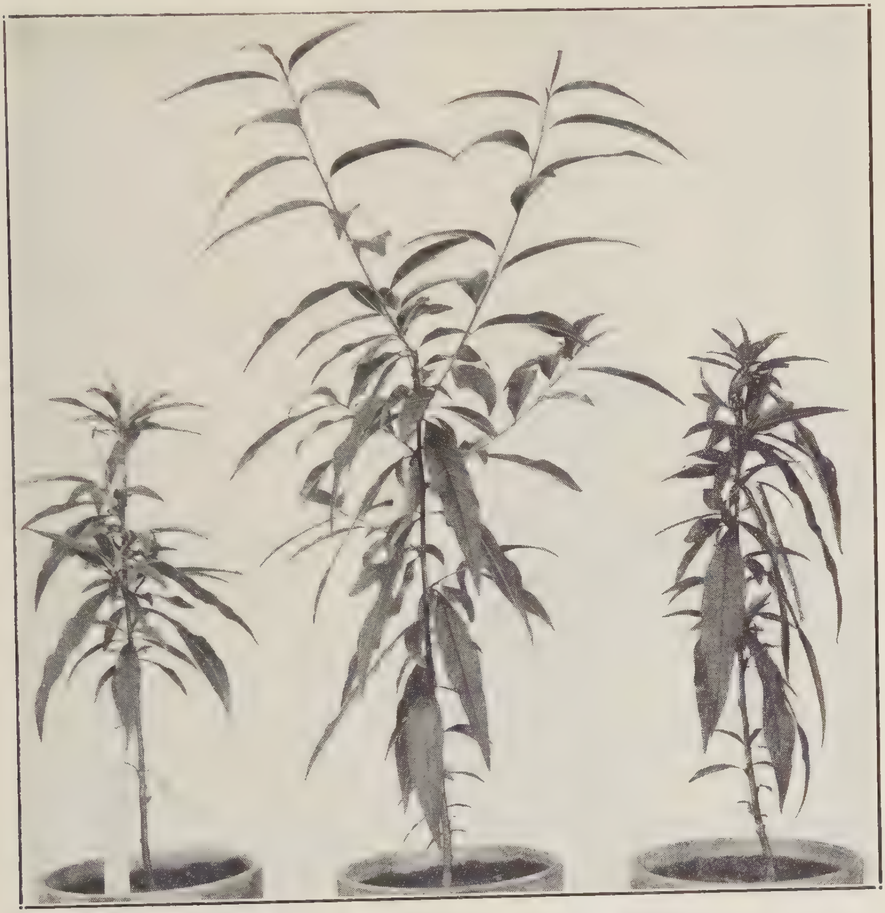

FIG. 2. Two diseased and one check peach seedling showing the stunting or rosette symptom typical of what obtained four to six weeks after inoculation when the rapid transmission technique was used for indexing cherry yellows buds on peach.

Disease symptoms were beginning to appear on the 21st day, and all the trees receiving buds from diseased cherry trees developed the typical symptoms of chlorotic followed by necrotic rings and rosette. Negative results were obtained in the 2 cases with doubtful material and in the 4 checks. A typical rosette condition at the end of 4 weeks is shown in figure 2. Note the difference in length of growth between the diseased trees and the check.

Experiment 4. In experiment 4 the budwood was collected from 6 different cherry orchards in August and tested on small peach seedlings, as in experiment 3, with similar results (100 per cent infection). Three trees were budded from each of 6 diseased and 1 healthy sources. Pruned uninoculated check trees also were included. The plants were budded on August 19, pruned back to 1 node above the diseased bud 5 days later, and were showing the characteristic early symptoms of chlorotic ring spot and rosette within 3 weeks. The plants receiving the buds from the healthy source grew normally. All yellows buds used in this experiment came from known severely diseased trees. While removing the leaves long pedical stubs were left to mark the location of the yellow leaves on the bud sticks, and buds from these locations were used exclusively.

Experiment 5. The fifth indexing experiment tested 3 sources of diseased buds from orchards each budded on 5 different seedlings with results practically identical to those obtained in experiment 4. The 5 trees receiving healthy buds were negative.

Experiment 6. The final indexing experiment involved budding 2 trees each from 2 sources—1 diseased and 1 healthy—and pruning immediately afterward. The buds were inserted on September 18 and the first symptoms, consisting of chlorotic rings and rosette, were evident 17 days later. Pruning immediately after budding seemed definitely to have shortened the incubation period in this experiment. One of these diseased seedlings, photographed at the end of 3 weeks, showed typical early stage symptoms (Fig. 1, C).

Thus, by means of the rapid transmission technique, it has been found possible to collect cherry budwood from trees showing yellows symptoms and to index and, therefore, certify as to the presence or absence of cherry-yellows virus within a month's time.

Because of its rapidity this technique has made it possible to select sources of propagation material in early summer and then to index same so as to obtain results before the regular budding season late the same summer.

After the initial chlorosis, which usually disappears, the severe stunting or rosette condition ordinarily persists throughout the remainder of the season (Fig. 1, B). However, there were exceptions in which the initial symptom stages were followed by a partial recovery suggesting some variability in the virus itself. Infrequently some stunting occurred in occasional peach seedlings receiving buds from apparently healthy cherry trees. To further complicate the picture, stunting has also been induced in peach seedlings by several other cherry viruses now under investigation. It is obvious, therefore, that much additional work will be needed to clarify the broader problem of cherry viruses.

Apparently the rapid transmission technique has definitely demonstrated its reliability for identifying severely diseased trees, but it will remain for future studies to determine its accuracy and reliability with cherry trees in the early stages of disease.

This technique, because it is not exacting for materials, space, or time, should prove generally useful for greenhouse studies, and probably for outdoor work, with proper care and attention to details. It also has possibilities in studies on other viroses (2).

SUMMARY

The cherry-yellows virosis has been transmitted by budding and grafting from diseased to healthy cherries with the production of definite yellow-leaf symptoms within approximately 1 year.

It also has been transmitted by budding in late summer from diseased cherries to healthy peach seedling trees with the production of dieback and a stunting rosette condition the following growing season. When the shoot above the bud was removed from 4-year-old Elberta peach trees, about a week after budding, instead of inducing new shoot growth the stub died back several inches below the diseased bud within a month from the pruning operation.

In the first indexing experiment, which involved placing diseased cherry buds between two rapidly growing shoots on cutback second-year Elberta peach grafts, chlorotic ring-spot symptoms were induced within 2 weeks from budding.

Buds from cherry trees affected with yellows indexed on yearling peach seedlings and cut back to 1 node above the diseased bud, resulted in the production of symptoms consisting of ring spot and rosette within a month from budding.

When repeated on small 18-inch-tall seedlings, shorter incubation periods of about 3 weeks were obtained in 4 different experiments.

This rapid indexing technique offers promise in advancing the work on cherry yellows and, in some modification, also may prove generally useful in studies on other stone-fruit viruses.

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AN UNUSUALLY VIRULENT RACE OF WHEAT STEM RUST, NO. 189

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(Accepted for publication December 18, 1941)

A hitherto undescribed race of *Puccinia graminis tritici*, extraordinarily interesting and important in several respects, has been identified from rusted Khapli emmer collected in Peru. This race, numbered 189 in the key of Stakman and Levine (7, 8), can cause heavy infection on all the differential wheat varieties used in identifying races. No other known race has this ability, the nearest approach being race 15, which attacks all but Khapli emmer. Four other races, Nos. 41, 42, 72, and 99, can cause moderate to rather heavy infection on seedlings of Khapli, but only under very favorable environmental conditions. Race 72 causes a type-X infection, indicating a considerable range in size of uredia; race 99 produces type 3, which indicates moderate susceptibility; and races 41 and 42 produce type 3 to 4, usually with pronounced chlorosis or even a tendency to necrosis around the uredia. None of these 4 races have been found in nature in the United States or Mexico. Race 41 was isolated from Egypt; race 72 has been obtained only from artificially made crosses in the greenhouse; and race 99 has been isolated only from South Africa, by Verwoerd (12). Race 42 was first isolated by Stakman and Levine from rusted wheat grown in Egypt and was subsequently found by Vallega (10) to be rather common in Argentina and neighboring regions. In studying factors affecting the development of this race on Khapli, Vallega found that it caused a wide range in infection type, both on seedlings and adult plants, depending on environmental conditions; it causes really heavy infection only under optimum temperature and light conditions. Race 189, on the other hand, does not require especially favorable conditions but can attack seedlings and adult plants of Khapli heavily under a rather wide range of conditions. It also can attack seedlings and adult plants of *Triticum timopheevi*, hitherto resistant to virtually all races, and is quite virulent on Hope wheat, one of the most generally resistant of all adequately tested varieties.

That race 189 is of practical importance is shown by the fact that Khapli emmer, grown commercially for a number of years in coastal regions of Peru because of its resistance to stem rust when other varieties tested were susceptible, has been very severely injured by rust for at least two successive

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[1] The experiments on which the results reported in this paper are based were made cooperatively by G. Garcia-Rada, of the Estacion Experimental Agricola de La Molina, Lima, Peru; J. Vallega, of the Instituto Fitotecnico, Llavallol, F. C. S., Argentina; and members of the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, cooperating with the Minnesota Agricultural Experiment Station.

The writers are indebted to Dr. M. N. Levine and Dr. E. A. Ausemus for help in obtaining some of the data for the paper.

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years. Several years ago Abbott (1, 2, 3) stated that Khapli emmer and Hope wheat might rust heavily in Peru. He concluded that a new and unusually virulent race of *Puccinia graminis tritici* was present, as all the differential varieties of Stakman and Levine were susceptible when inoculated in the greenhouse with rust from Khapli emmer. Several attempts were made later to identify this Peruvian rust at the Federal rust laboratory, St. Paul, Minn., but the spores were nonviable in all the collections obtained. In 1939 one of the writers (Garcia-Rada) called attention to the fact that Khapli had been severely injured by rust in certain wheat-growing localities in the coastal region of Peru (Fig. 1). In the same year he sent uredial material to Vallega at the Instituto Fitotecnico at Llavallol, F. C. S., Argentina, and to the Federal rust laboratory at St. Paul, Minn. Infection was obtained with the material sent to Argentina, but spores of the sample sent to St. Paul were not viable on arrival. The infection types produced on the differential varieties by the rust sent to Argentina were as follows:[2]

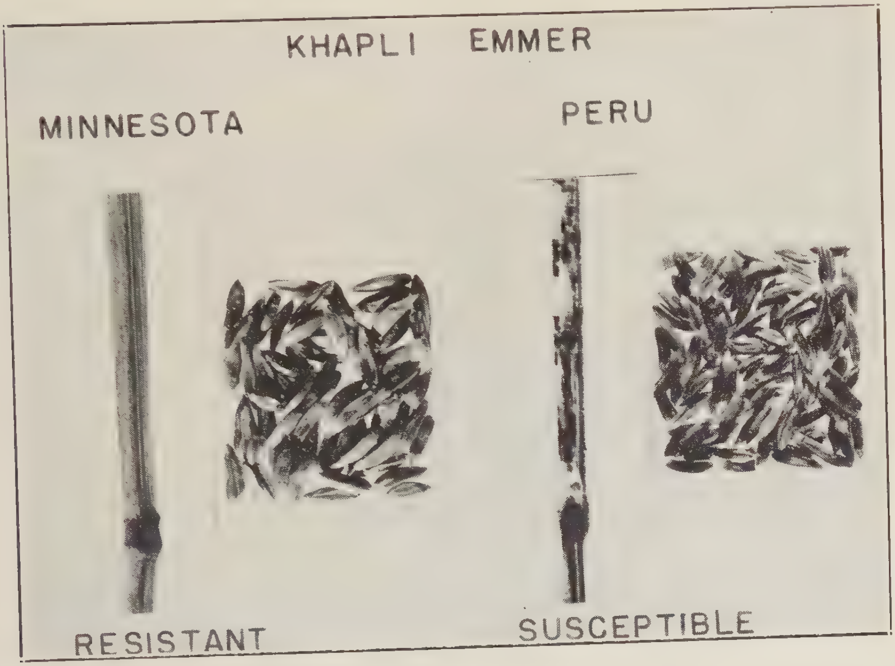

FIG. 1. Khapli emmer, resistant to rust in Minnesota (left) and damaged by rust in Peru (right).

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[2] In the system of Stakman and Levine (7), infection types range from 0 to 4, 0 indicating immunity, 1 and 2 indicating resistance, and 3 and 4 indicating susceptibility. The uredia in type 4 are larger than those in type 3 and have a greater tendency to coalesce. Sometimes there are imperceptible gradations between types, especially types 3 and 4, and the deviations from the standard of the type are shown by plus or minus signs. The letter c simply shows that there were pronounced chlorotic areas around uredia. There is a sixth type, designated X, in which individual uredia represent a considerable range of types.

Hood[a]	4	Spelmar	4
Marquis	4	Kubanka	4
Reliance	4	Acme	4
Kota	3 +	Einkorn	4
Arnautka	4	Vernal	4
Mindum	4	Khapli	4[c]

[a] Substitute for Little Club.

It was evident from these infection types that the collection comprised a new physiologic race (11). In September, 1940, one of the writers (Stakman) visited the experiment station at Lambayeque, Peru, at the suggestion of Garcia-Rada, and was shown heavily rusted plots of Khapli emmer, Hope wheat, *Triticum timopheevi*, and several other varieties that had been highly resistant for a number of years in many widely separated geographic regions. Some of the rusted Khapli was sent by airmail to St. Paul, Minn., through the Division of Foreign Plant Quarantines, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, and inoculations were made later when there was no danger of spores being disseminated to growing vegetation. A pure culture was obtained of a race that attacked all the differential varieties heavily, thus agreeing with Vallega's results and confirming Abbott's earlier conclusion regarding the existence in Peru of an unusually virulent race, to which the number 189 has now been assigned (Fig. 2).

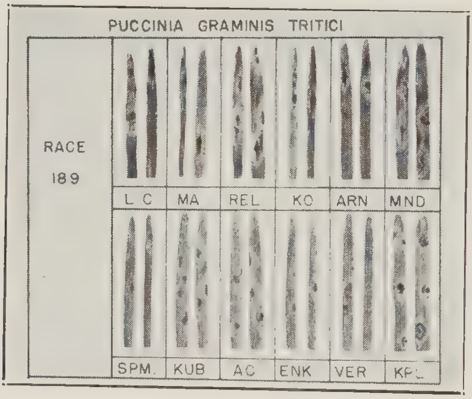

FIG. 2. The effect of race 189 on the differential host plants.

Although the work with this collection was done during the dormant season, so that there was no opportunity for spores to be blown to growing vegetation, every possible precaution was taken between the arrival of the collection and the time when it was completely discarded. All inoculated plants and the soil and pots in which they were grown were disinfected in a large steam sterilizer under 15 pounds of pressure for 2 hours. Inoculations were made in an isolation booth, and the greenhouse benches, partitions, and walls of the isolation booth were dusted with sulphur and later washed with copper sulphate solution.

Race 189 is very virulent not only on seedlings in the greenhouse but also on adult plants in the field. Seedlings of about 50 resistant varieties and selections recently produced in the United States were at least moderately susceptible when inoculated in the greenhouse. More important, however, is the ability of race 189 to cause heavy damage, in the field, to adult plants of varieties such as Khapli, Hope, and *Triticum timopheevi* that have adult-plant resistance in addition to their protoplasmic resistance to many physiologic races. As far as the writers are aware, Khapli, for example, has not been rusted heavily anywhere except in Peru, where in some years and localities it would have been considered a completely susceptible variety by any one unfamiliar with its record elsewhere. It is true that weather conditions for rust development are likely to be favorable in the coastal region of Peru because of the abundance of fog; but the most important factor in the susceptibility of Khapli and other hitherto resistant varieties seems to be the presence of race 189.

Stakman and Hart (6) emphasized the fact that the resistance of wheat varieties to stem rust, especially in the field, is determined by a complex series of interacting factors, genetic and environmental, involving the host, the particular race of the pathogen, and the interaction between the two. It is evident that certain otherwise resistant varieties cannot withstand the combination of a very virulent rust race and favorable rust weather in at least part of the coastal region of Peru. Possibly it is futile to attempt to assess the relative responsibility of the weather and of the pathogen. Obviously, even so virulent a race as 189 requires favorable weather for infection and subsequent development, but even the most favorable weather will not enable nonvirulent races to attack resistant varieties. Khapli emmer, for example, has remained resistant in the United States from 1914 to 1941, inclusive, even in the most devastating natural and artificially induced stem rust epidemics.

Khapli was introduced into the United States from India in 1908 (4), has been tested in certain experimental plots and rust nurseries for about 30 years, and for about 20 years has been grown in the uniform rust-observation nurseries of the U. S. Department of Agriculture at an average of about 50 stations in different regions of the country. Furthermore, there are records of its performance in or adjacent to the rust-breeding nursery at University Farm, St. Paul, Minn., each year from 1914 to 1941, inclusive.[3] In this nursery, epidemics are induced artificially by inoculating with most of the races of wheat stem rust that occur in nature in the United States. And yet the rust readings on Khapli, even under such exceptionally severe conditions, have been "trace" every year except 1916, 1922, 1925, and 1926. In 1916 the reading was 50 ± per cent, while that for the susceptible Little Club was 95 per cent; in 1922 the reading was 10R, and in 1925 and 1926 it was 5R. The figures represent the percentage of what is considered the

[3] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Division of Agronomy and Plant Genetics and Division of Plant Pathology and Botany, Minnesota Agricultural Experiment Station.

maximum possible number of pustules, and "R" indicates "resistant." In only one year of the 28, then, was there a really appreciable amount of rust on Khapli. In 1916, when one of the worst epidemics on record developed in the spring-wheat region of the United States, there were about half as many pustules on Khapli as on the most susceptible varieties, but they were small.

One of the writers (Stakman) has studied and observed Khapli for more than 30 years. It was one of the varieties studied in early attempts to learn something about the nature and variability of resistance (5) and is one of the differentials used in identifying physiologic races. During the past 20 years seedlings of Khapli have been inoculated with rust from more than 10,000 collections of wheat from the United States and Mexico, but have been highly resistant to every one of them. Khapli has therefore been inoculated with all physiologic races identified during that time but has not been susceptible, even in the seedling stage, to any race ever found in nature in the United States and Mexico. Furthermore, the same writer has seen Khapli in field plots at many widely separated stations in many different years in the United States but has never seen large pustules or evidences of injury. Environmental conditions certainly have been favorable for the development of rust in the thousands of tests in the greenhouse, because susceptible varieties rusted heavily; conditions were favorable for rust development in the rust nursery at St. Paul, Minn., where there has been an epidemic, artificially induced and fostered, each year for many years; and there have been several devastating and widespread natural epidemics and a number that were destructive locally. Obviously, then, Khapli has been very extensively tested against North American rust races, often under ideal conditions for rust development, and has been one of the most highly and consistently resistant, along with Hope and *Triticum timopheevi*, of all varieties tested.

The complete susceptibility of Khapli in Peru, therefore, appears to be due primarily to the prevalence of the especially virulent race 189, and the same appears to be true of Hope wheat and *Triticum timopheevi*. This supports the conclusion that, although some varieties of wheat have a combination of factors for resistance that enables them to withstand destructive attacks of stem rust in most seasons and in most regions, nearly all known varieties may sometimes become more or less heavily rusted by some rust races, in some regions and under certain combinations of environmental conditions. Consequently the importance of continuing the attempt to find and combine the most generally effective genes for resistance to stem rust seems obvious.[4]

Race 189 has been found only in Peru, where it appears to be distributed in the coastal region from Lambayeque on the north to Lima on the south.

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[4] It appears now that a few hybrid selections are at least partly resistant in Peru, even in the presence of race 189, the most generally virulent race of wheat stem rust now known; but further tests are needed, and, in any case, a detailed account of varietal behavior is beyond the scope of the present paper.

Whether it is more widespread in Peru is not known. It has not yet been found elsewhere in South America and has never been isolated either from wheat or barberry bushes in the extensive physiologic-race surveys that have been made annually in the United States for about 20 years. It seems unlikely that it would have been missed had it been present even in small amounts. Supporting evidence that it does not occur in North America is the fact that Khapli emmer has been resistant for about 20 years in approximately 50 uniform rust nurseries in widely separated places in the United States.

Whether urediospores of this very virulent and dangerous race can be carried by the wind from Peru to other countries of South America or to North America is an important question. If it is now restricted to the coastal region of Peru, the Andes mountains constitute a formidable but not necessarily impassable barrier to its eastward spread. As the present distribution seems to be south of the equator, the likelihood that inoculum might be blown to North America may seem somewhat remote, although it is possible that the rust might spread northward across the equator by easy stages on grains or grasses at high elevations and become established in northwestern South America, from which spores might possibly be carried by the wind to Central America, Mexico, and grain-growing regions of the United States and Canada. In any event it seems important to ascertain the extent to which urediospores are disseminated by the wind in equatorial regions and particularly whether they can be carried from one continent to another.

Nothing is known regarding the time and mode of origin of race 189. There is circumstantial evidence that it has been present in Peru for at least 15 or 20 years, possibly longer. New races of *Puccinia graminis* are known to result from recombinations in the sexual stage on *Berberis* spp., the alternate hosts of the rust. Native barberries are numerous in certain parts of the Sierra of Peru and they are now known to become infected. Although they may not be important in initiating annual outbreaks of rust, it seems certain from experience elsewhere (9) that they probably are important in the perpetuation of physiologic races and the production of new ones. Race 189 never has been isolated from the aecial stage on barberries in North America nor has it been obtained from artificial crosses. Possibly the combination of genes represented in this race is rarely made. On the other hand, it has been made and may be made again. At all events, the existence of so virulent a race is definite confirmation of the statement so often made in the past that nature can produce very virulent races of rust just as man can produce very valuable varieties of wheat. Therefore, it is important to study the prevalence and distribution of physiologic races, eradicate the alternate host where feasible, and continue breeding work to find and combine genes for resistance in the most effective manner possible.

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A STEM–END ROT OF POTATO TUBERS CAUSED BY RHIZOCTONIA SOLANI[1]

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(Accepted for publication December 5, 1941)

Rhizoctonia solani is widely considered to be a species comprised of many physiological races, and is recognized as a parasite of an unusually extensive host range. Nevertheless, reports of the decay of normal potato tubers by this organism are uncommon. Shapovalov[3] has described a "stem-end decay of the jelly type" caused by *Rhizoctonia solani*, but in this instance each affected tuber was one with an abnormally elongated stem-end, the decay being confined to this region, which was characterized by "a striking deficiency in starch." Since preparation of this paper, Storey[4] mentions having isolated *R. solani* from a "tuber showing jelly-end-rot," but was unable to duplicate symptoms by artificial inoculation.

Potato tuber specimens showing a stem-end decay of a "punky" consistency were submitted recently to the writer from a locality in Prince Edward Island. *Rhizoctonia solani* was isolated from these specimens and its pathogenicity proved by inoculation tests.

The disease apparently originated at the stolon scar, involving parenchyma tissue radially from that point for a distance of 1 to 3 cm., and was finally evident as a dark necrosis with an abrupt margin. The external appearance of the specimens was thus strongly suggestive of the stem-end rot caused by *Fusarium solani* var. *eumartii* (Fig. 1, A–C).

When first examined, the necrotic tissue was of a resilient, "punky" or "cheesy" consistency. On exposure to laboratory air the lesions soon became sunken and, whenever the periderm was broken, the diseased tissue dried out quickly and shrivelled.

The microscope reveals the typical brown hyphae of the pathogen dispersed among the cells of the diseased parenchyma tissue. Ultimately, the progress of the fungus is restricted by extensive phellogen formation in the healthy tissue just beyond the margin of the necrotic zone (Fig. 2, A and B). The phellogen, and the suberized cells developing from it, are not always effective as a barrier, in which case the lethal action of the fungus continues until another phellogen layer surrounds the secondary necrotic region (Fig. 2, B). In the specimens examined the cell walls of the more recently invaded tissues were intact, but necrotic cells a few cell diameters away from the border of the affected region had undergone cell wall disintegration to such an extent that the residuum was little more than a mass of starch grains, of which there was the normal complement. There was no indication that the fungus induced hydrolysis of starch grains, as is evident in figure 2, D. Figure 2, A and B, illustrate clearly the disintegration of cell walls, which is the change that probably accounts for the "punky" nature of the necrotic mass.

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[1] Contribution No. 687 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Junior Plant Pathologist.

[3] Shapovalov, M. *Rhizoctonia solani* as a potato-tuber rot fungus. Phytopath. 12: 334–336. 1922.

[4] Storey, I. F. A comparative study of strains of *Rhizoctonia solani* (Kühn) with special reference to their parasitism. Ann. Applied Biol. 28: 219–228. 1941.

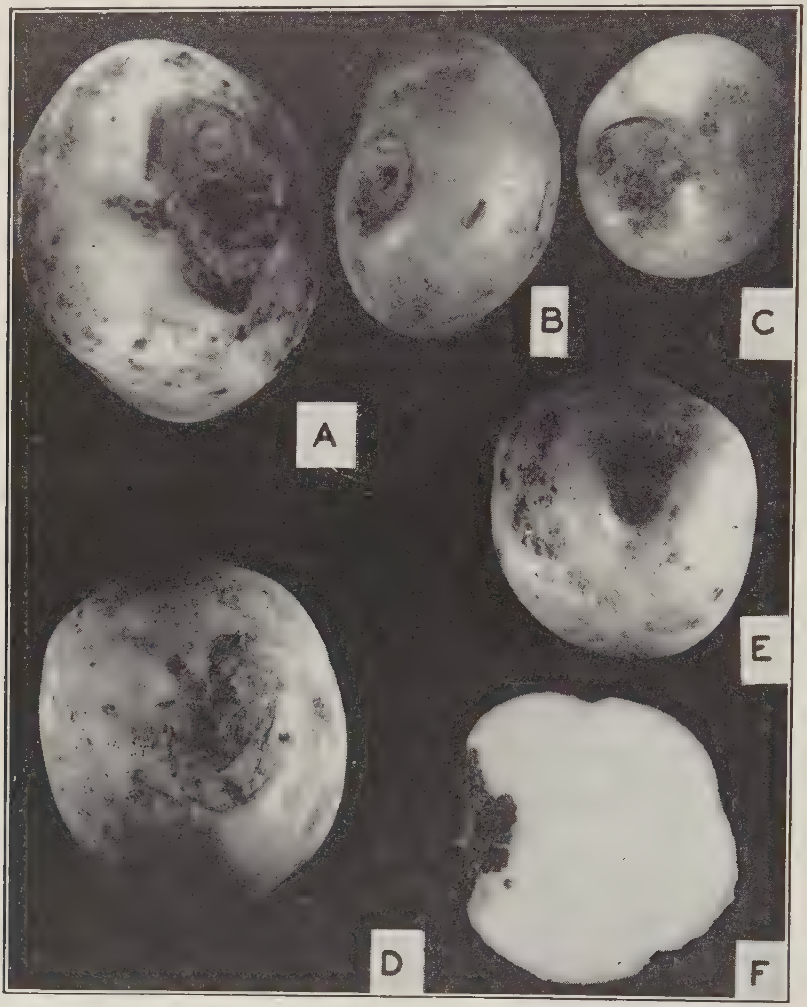

FIG. 1. A–C. Stem-end decay caused in the field by *Rhizoctonia solani.* × ⅔. D–F. Stem-end decay caused by *R. solani* following artificial inoculation; tuber kept in wet sand at 15–20° C. after inoculation. E. Tuber held at 5° C. after inoculation.

Sections from tissue embedded in paraffin reveal that the tracheids are the probable path of entrance of the pathogen. Hyphae were found to be plentiful within the lumina of tracheids present in the vascular bundles leading from the stolon scar, the hyphae sometimes being detected in the tracheids in tissue beyond any visibly necrotic parenchyma. At intervals along the path of the bundle the hyphae escape from the tracheids *via* pits

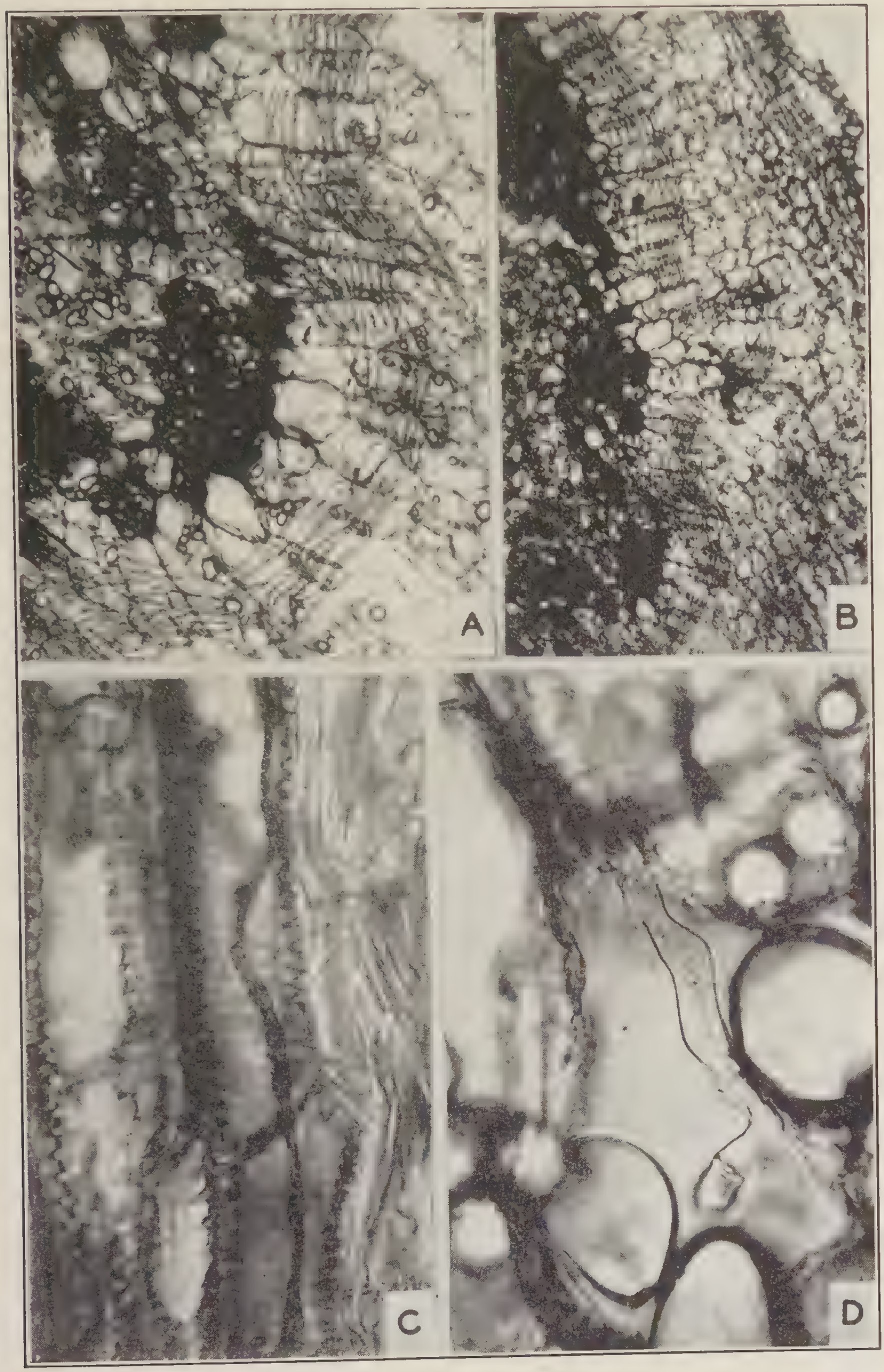

FIG. 2. Sections of potato tuber tissue infected by *Rhizoctonia solani*. A. Extensive phellogen development surrounding necrotic tissue. × 100. B. Necrotic tissue surrounded by a phellogen, which proved to be an ineffective barrier to the fungus. The small region of secondary decay is being surrounded by another phellogen layer. × 40. C. Hyphae of *R. solani* present in the lumina of tracheids. × 1,000. D. Starch grains in decaying tissue which show no sign of being hydrolyzed by the fungus. × 1,500. A, B, and D. Freehand, unstained sections. C. Paraffin section, stained by safranin and fast green.

into parenchyma cells, whence typical cell degeneration occurs. This explains the fact that the furthermost regions of disintegrating parenchyma are evident as short cones of necrotic tissue centered about groups of infected tracheids. *Rhizoctonia* mycelium within the lumen of a tracheid is seen in figure 2, C.

Very wet and cool weather prevailed just before harvest in the locality in which the diseased specimens had been found. Under these conditions of low temperature and low oxygen-tension (the latter brought about by the soil being saturated with water), a wound periderm is formed very slowly at the stolon scar and the ends of the vascular bundles leading from the stolon are not effectively occluded. Hence, the *Rhizoctonia* hyphae are able to grow from the decaying stolon into the tuber tissues and are able to continue growth until such time as conditions favor wound-periderm formation. The appearance of the specimens seemed to indicate that this was not complete at time of digging.

The results from pathogenicity tests support the suggestion that *R. solani* can cause decay in the potato tuber unless prevented by rapid wound-periderm formation. Green Mountain tubers that had been in storage for a month were inoculated by inserting mycelium from a 2-week-old culture growing on potato-dextrose agar into a small incision made at the stolon scar. Some of the tubers were kept in moist chambers held at temperatures of 5, 14, and 20 degrees C.; others were covered in very wet sterile sand, kept at from 15° to 20° C. Typical symptoms developed in the tubers held at 5° C. and in those kept in the wet sand (Fig. 1, D–F). Under each of these conditions periderm formation would be slow. Tubers in the moist chambers held at 14° and 20° C. developed only a slight degree of "Stem-end browning."

FURTHER STUDIES ON THE TEMPERATURE RELATIONS OF SCLEROTIAL ISOLATES OF RHIZOCTONIA SOLANI FROM POTATOES[1]

E. L. LeClerg, L. H. Person, and S. B. Meadows[2]

(Accepted for publication March 13, 1942)

INTRODUCTION

Previous investigations[3] on the relation of temperature to radial growth on artificial media of 20 isolates of *Rhizoctonia solani* Kühn from sclerotia on potato tubers indicated an optimum at 25° C. or lower. Under identical conditions, the optimum temperature for 19 of 20 crown-rot isolates from sugar beets was 30° C., one isolate growing slightly faster at 25° C. In further work,[4] it was found that the optimum temperature for radial growth of dry-rot isolates from sugar beets was also 30° C., whereas it was 25° C. for 4 additional sclerotial isolates from potato tubers.

One of the writers (Person) had occasion to isolate this fungus from sclerotia on a large number of potato samples from several States producing certified-seed. These isolates formed the basis for this study, which was made to supplement the investigations mentioned above.

EXPERIMENTAL RESULTS

The influence of temperature on radial growth of 63 sclerotial isolates from potatoes, two crown-rot isolates from sugar beets, and 2 dry-rot canker isolates from sugar beets was studied at 20, 25, and 30° C. The data presented are averages of duplicate tests each of which consisted of 2 cultures on potato-dextrose agar at each temperature. Agar discs, of a uniform size cut out with a sterilized cork borer, were used for inoculum.

From the data in table 1, it appears that 25° C. was the optimum temperature for all of the potato sclerotial isolates. The optimum temperature for the sugar-beet crown-rot and dry-rot isolates, however, was 30° C. These results confirm those previously reported.[5]

In making isolations of sclerotia from tubers more than one sclerotium from a tuber was planted on agar. In table 2 is indicated the radial growth on potato-dextrose agar of two isolates from each of four potato tubers at 20°, 25°, and 30° C. It is apparent that sclerotial isolates from the same tuber may differ in rate of radial growth at the several temperatures.

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[1] The data herein presented were obtained in cooperative investigations by the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture and the Departments of Botany, Plant Pathology, and Bacteriology, and of Horticultural Research of the Louisiana Agricultural Experiment Station.

[2] Pathologist of the Division of Fruit and Vegetable Crops and Diseases, United States Department of Agriculture, Assistant Pathologist of the Louisiana Agricultural Experiment Station, and Agent of the Division of Fruit and Vegetable Crops and Diseases, United States Department of Agriculture, respectively.

[3] LeClerg, E. L. Comparative studies of sugar-beet and potato isolates of *Rhizoctonia solani.* Phytopath. 31: 274–278. 1941.

[4] LeClerg, E. L. Studies on dry-rot canker of sugar beets. Phytopath. 29: 793–800. 1939.

[5] See footnotes 3 and 4.

TABLE 1.—*Optimum-growth temperature for isolates of Rhizoctonia solani from sclerotia on potatoes and from crown-rot and dry-rot lesions of sugar beets, as indicated by the number of isolates with maximum radial growth at the indicated temperature*

Source	Number of isolates tested	Number of isolates with optimum temperature at		
		20° C.	25° C.	30° C.
Sclerotial isolates from potatoes				
Nebraska	38	0	38	0
North Dakota	18	0	18	0
Maine	4	0	4	0
Colorado	2	0	2	0
Michigan	1	0	1	0
Crown-rot isolates from sugar beets				
Michigan	1	0	0	1
Ohio	1	0	0	1
Dry-rot isolates from sugar beets[a]				
California	1	0	0	1
Ohio	1	0	0	1

[a] The writers are indebted to Dr. J. E. Kotila for the dry-rot isolates from sugar beets.

TABLE 2.—*Comparative radial growth at 3 different temperatures of two isolates of Rhizoctonia solani obtained from different sclerotia on each of four potato tubers*

Source of tuber	Tuber number	Average colony diameter (mm.)		
		20° C.	25° C.	30° C.
Nebraska	2a	45.0	56.0	50.2
	2b	36.2	46.5	23.5
Nebraska	32a	46.0	58.2	46.2
	32b	60.5	62.5	44.0
North Dakota	5a	55.7	62.2	46.5
	5b	55.0	57.5	43.0
North Dakota	12a	56.2	61.2	44.7
	12b	47.5	54.0	51.7

SUMMARY

Comparative studies were made to determine the optimum temperature for radial growth on potato-dextrose agar of 63 isolates of *Rhizoctonia solani* from sclerotia on potato tubers. Two crown-rot and 2 dry-rot canker isolates from sugar beets also were used. Determinations were made at 20°, 25°, and 30° C.

It was found that potato sclerotial isolates made the greatest radial growth at 25° C. The isolates from the two types of sugar beet lesions grew best at 30° C. These results confirm those previously reported by the senior writer.

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PRELIMINARY INVESTIGATIONS ON MECHANICAL INJURY IN FLAX SEED[1]

J. E. Machacek and A. M. Brown

(Accepted for publication March 4, 1942)

An examination of a large number of flax-seed samples in 1940 revealed that practically all of those originating in Manitoba, Saskatchewan, and Alberta were so damaged through a cracking of the seed that, on the average, about half the seeds failed to germinate. Seed samples from Ontario, Quebec, and the Maritime Provinces usually were not injured at all. In 1941, there was less cracking of the seed, but the amount of damage was again greater in Western than in Eastern Canada. This injury to flax appeared to be similar to that described, respectively, by Stevens[2] and by Härtel[3] for the United States and Germany.

The cracking of the seed was observed to occur during threshing, the available evidence indicating that the damage was likely to be most severe when the crop was threshed in very dry weather. Ordinarily, the cracks were invisible to the unaided eye, but sometimes their presence was indicated by broken or chipped seeds. Many samples, grading high in commerce, were found to be badly damaged. Large-seeded varieties seemed to be more subject to injury than were small-seeded varieties.

When samples containing cracked seeds were planted in the greenhouse in beds of nonsterile soil, such seeds either failed to germinate or produced stunted, distorted plants, thus causing a reduction in stand, which seemed attributable to the rotting of cracked seed by soil-inhabiting micro-organisms. The rotting did not occur when the seed was germinated on moist paper, as in official tests, or in autoclaved soil. Soil temperature within the range of 10–30° C. appeared to have no effect on the amount of seed rotting in nonsterile soil, but the rotting was less when the seed was sown in light soils than in heavy soils.

Disinfection of the seed with Ceresan or Half-ounce Leytosan completely prevented the rotting of cracked flax seed in nonsterile soil. The most effective rates of application for these dusts were found to be 1½ ounces per bushel for Ceresan and 2 ounces per bushel for Half-ounce Leytosan. The average germination, for 362 flax samples collected in 1940 and 1941, was about doubled by this treatment. In seedlings from cracked but treated seed, the only evidence of former injury to the seed was found on the cotyledons, which were scarred where the seed had been cracked.

Field experiments, in 1941, with 8 varieties of flax obtained from each of

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[1] Contribution No. 697 Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Stevens, O. A. Germination studies on aged and injured seeds. Jour. Agr. Res. [U. S.] 51: 1093–1106. 1935.

[3] Härtel, K. Über druschverletzten Lein und keimverletztes Getreide und ihre Beurteilung bei der Reinheitsbestimmung. Compt. Rend. Assoc. Internat. d'Essais de Semences 2: 213–223. 1936.

6 different stations showed that the cracking of the seed influenced the density of stand in the same way as it did in the greenhouse. The reduction in stand was proportional to the amount of seed cracking. The planting of cracked seed also reduced the yield of grain, but the amount of this reduction seemed to be affected by the location of the experiment. At Winnipeg, for instance, yields were not reduced when less than 20 per cent of the seeds were cracked. At Morden, Manitoba, where growing conditions for flax were less favorable than at Winnipeg, this amount of cracking reduced the yield. In both cases, the disinfection of cracked seed raised the yield to the level in the crop from undamaged seed. To illustrate the effect of seed disinfection on cracked seed, the results obtained from duplicate experiments at Winnipeg and Morden are shown in table 1. In these experiments, 3 different seed lots of Redwing flax were sown in 1/100-acre plots arranged in a Latin square. Full competition from weeds was allowed.

TABLE 1.—*The effect of seed disinfection with Ceresan (1½ ounces per bushel) on seed germination and yield in three lots of flax seed showing different degrees of mechanical injury*

Seed sample	Percentage increase from seed treatment				
	Germination			Yield	
	Laboratory	Field (Winnipeg)	Field (Morden)	Winnipeg	Morden
A	41.0	17.1	58.3	0.0	20.0
B	91.0	33.1	85.8	6.0	33.5
C	174.0	38.9	92.2	12.5	45.5

The results of this preliminary investigation suggest that, in comparative tests of varieties, it is important to plant undamaged seed or, if the seed is damaged, treated seed. In comparative tests of disinfectants, either mechanically-injured or diseased seed should be used. A sample of seed of a desirable variety may be sufficiently damaged to lower its ordinary yield, while in tests with seed disinfectants, the use of different lots of seed is likely to give conflicting results.

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PHYTOPATHOLOGICAL NOTES

A Blue-staining Fungus Inhabiting the Heartwood of Certain Species of Conifers.[1]—There are few records of bluestain in the heartwood of living trees. Lagerberg, Lundberg, and Melin[2] reported stain in the heartwood of a single living spruce tree (*Picea excelsa*). They isolated the causal organism and on the basis of cultural characteristics and the formation of small hyaline spores described it as a new species of *Hormodendrum, H. microsporum* (Lagerberg and Melin). Kaufert,[3] in 1935, reported bluestain in the heartwood of approximately 10 per cent of overmature balsam fir trees, the fungus apparently entering through dead branch stubs. Crowell[4] later described a dark bluestain occupying most of the heartwood of a single specimen of white spruce and one of balsam fir, and he stated that when pieces of wood were kept in a moist chamber for 5 weeks, mycelium grew out of the stained wood but formed no spores. His excellent illustration leave little doubt that he was dealing with the same fungus previously mentioned by Kaufert and discussed in the present note. Scheffer and Lindgren[5] state in a footnote that, ". . . also certain uncommon bluestains have been encountered developing only in the heartwood of living trees."

During the last few years the writers have cut and dissected more than 50 northern white cedar trees (*Thuja occidentalis*) in the forests of Northern Minnesota, and have found bluestain in the heartwood of every one. It usually is in streaks above and below branches or branch stubs, and sometimes occupies a considerable volume of the heartwood. Numerous isolations from the stained wood have in almost every case yielded a non-sporulating, very slow growing fungus with dark hyphae, which is the organism chiefly or solely responsible for the stain in the wood, plus one or more sporulating fungi, as yet unidentified. Typically, when isolations are made on malt- or potato-dextrose agar, one sees macroscopically only the dark, slow-growing fungus. Macroscopically, the culture looks pure. In more than a hundred isolations made on different agar media the bluestaining fungus never has been obtained alone, but always in association with other organisms. Pure cultures of the stain fungus were obtained by cutting hyphal tips on hanging agar drops. Approximately 20 cultures made in this way were very similar to each other and only slightly different from the original cultures containing the two or more organisms.

Pieces of white cedar heartwood were sterilized in an autoclave and inoculated with pure cultures of the stain fungus. The fungus grew over the surface of and through this wood somewhat slower than it grew on agar, staining the wood rather dark blue. The mycelium is not restricted to particular cells or tissue systems of the wood, but grows through tracheids and rays much as do wood-rotting fungi. It can be found as abundantly in tangential as in radial sections. The hyphae are constricted as they pass through the cell walls. Its growth in wood is shown diagrammatically in figure 1. Hyphae of the associated fungus or fungi are somewhat less abundant than those of the stain fungus, but can be seen readily and grow through the wood in a similar way.

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[1] Paper No. 1992 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Lagerberg, T., G. Lundberg and E. Melin. Biological and practical researches into blueing in pine and spruce. Svenska Skogsvardsfor. Tidskr. 25: 145–272, 561–739. 1927–28.

[3] Kaufert, F. H. Heartrot of balsam fir in the Lake States with special reference to forest management. Minn. Agr. Exp. Stat. Tech. Bull. 110. 1935.

[4] Crowell, Ivan H. Heart bluestain of white spruce and balsam fir. Pulp and Paper Magazine of Canada 41: 451–452. 1940.

[5] Scheffer, T. C., and R. M. Lindgren. Stains of sapwood and sapwood products and their control. U.S.D.A. Tech. Bull. 714. 1940.

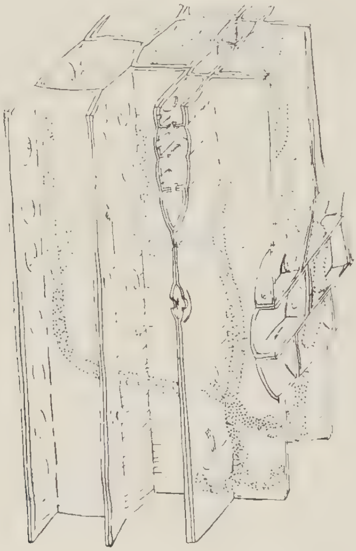

FIG. 1. Diagrammatic view of the bluestaining fungus in the heartwood of northern white cedar.

Although this stain fungus has not been observed to produce spores during several years in culture on various agar media and on wood, it can be disseminated rather readily by hyphal fragments. About 5 cc. of sterile distilled water were added to a culture 4 months old on an agar slant in a test tube. This was shaken twice, then poured over malt agar in 6 Petri dishes, the excess water being poured off, so that only little of the original 5 cc. remained on the agar. Approximately 1000 colonies of the fungus arose on the six plates. Attempts to dislodge similar fragments by merely shaking old cultures without adding water, or by inverting them above agar, were unsuccessful.

The stain fungus, with the associated organisms mentioned above, was obtained in about 20 per cent of several hundred isolations from a brown cubical trunk rot that originates at branch stubs, and from a brown feathery butt and trunk rot that originates in the roots, of northern white cedar. The same bluestain is found very commonly in branch stubs of northern white cedar and balsam fir and the same combination of organisms has been isolated from them repeatedly. What appears to be the same fungus has been observed in numerous samples of heartwood of western red cedar and Port Orford cedar.—C. M. CHRISTENSEN and F. H. KAUFERT, Division of Plant Pathology and Botany, Division of Forestry, University of Minnesota.

Cercosporella Eyespot of Kentucky Bluegrass.[1]—An undescribed disease, eyespot of Kentucky bluegrass (*Poa pratensis* L.), caused by a species of *Cercosporella*, occurs scatteringly in the Willamette Valley, Oregon. It was noted and specimens collected first at Forest Grove in March, 1939, and again a year later at East Corvallis. Strangely enough, although the symptoms are distinct, no specimens of it occur in any of the numerous earlier collections on this species of grass filed in the Mycological Herbarium of the Department of Botany at Oregon State College.

The writer has collected many specimens of *Septoria macropoda* Pass. var. **septulata** (Gz. Frag.) comb. nov. (*S. poae-annuae* var. *septulata* Gz. Frag.) on *Poa pratensis*, but in none of these does any of the cercosporella eyespot occur. It will be of interest, therefore, to note if this disease continues to spread under the cool, humid climate of mid-early spring in western Oregon.

On *Poa pratensis* the cercosporella eyespot is distinguished from the septoria leaf-spot and also from the leaf spot caused by *Helminthosporium vagans* Drecshl. as follows:

1. Cercosporella eyespot, lesions circular to sometimes finally elongate, brown with yellowish border, often straw colored in center and hence having an eye-spot type of lesion on at least some of the spots.
2. Septoria leaf spot, lesions variable in shape, rather uniformly ashy to stramineous in color, not an eyespot; pycnidia present.
3. Helminthosporium leaf spot, lesions circular to elongate with prominent, reddish-brown borders, darkening to nearly black in the center and, later, some becoming lighter in center.

The *Cercosporella* on *Poa pratensis* differs distinctly from any described species on Gramineae. Since the only possibility appeared to be the ill-described *Cercospora poae* Baudys et Picb., the writer sent material to Charles Chupp who very kindly examined it and replied that *C. poae*, in all probability, was the fungus known sometimes as *Scolecotrichum graminis* Fckl., and that the Oregon material probably was an undescribed species of *Cercosporella*. Chupp also suggested that it might be well to check on *Napicladium graminum* Peck. Material of this, furnished by H. D. House from the New York State Museum at Albany, also proved to be *Scolecotrichum graminis*. Therefore, *N. graminum* Peck can be added to the synonomy of *S. graminis* Fckl.

It appears, therefore, that the *Cercosporella* on *Poa pratensis* from Oregon is an undescribed species as follows:

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[1] Cooperative investigations between the Divisions of Cereal Crops and Diseases, and Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Oregon Agricultural Experiment Station. Published with the approval of the Director of the Oregon Experiment Station as Tech. Paper No. 393. Contribution from the Department of Botany.

Cercosporella poagena sp. nov.

Maculis brunneis, margine aureo, ellipticis v. orbiculatis, deinde elongatis brunneis v. stramineis; conidiophoris hyalinis, obscuris; conidiis elongatis, sub-filiformibus basis obtusatis, apicibis acuminatis v. attenuatis, 4–7 septatis, hyalinis 45–90 × 3.6–5 μ (fig. 1).

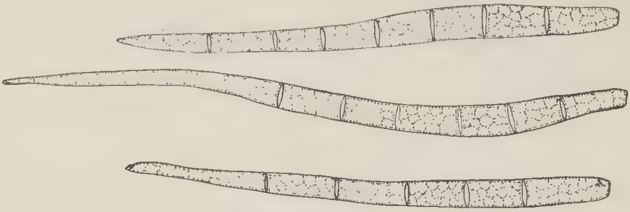

FIG. 1. Conidia of *Cercosporella poagena* sp. nov., from *Poa pratensis,* Forest Grove, Oregon (O.S.C. 453, type). × 1,000.

Hab. in foliis vivis *Poae pratensis,* Forest Grove, Oregon, March 15, 1939 (O.S.C. 453, type, filed at Oregon State College, Corvallis, Oregon, and a fragment of it in the Mycological Collections, Bureau of Plant Industry, Washington, D. C.). Also collected at East Corvallis (O.S.C. 789).

Lesions light-brown with a distinctive straw-color center and a surrounding border or halo of yellow; lesions at first very small, circular, later enlarging to nearly the width of the leaf or elongating longitudinally on the leaf for some distance; spots finally becoming dull brown and eventually fading to straw color. Conidiophores hyaline, obscure, arising from hyaline thinly formed aggregating hyphae; conidia hyaline, elongate, broadly filiform to obclavulate, bases blunt, tapering, apices either abruptly acuminate or attenuate at the tips, 4- to 7-septate (mostly 4-septate), 45–90 × 3.6–5 μ (45–75 ×3.6–4.6 μ in type and 57–90 × 4–5 μ in East Corvallis material).

On living leaves of *Poa pratensis,* Forest Grove (type) and East Corvallis, Oregon.—RODERICK SPRAGUE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Northern Great Plains Field Station, Mandan, North Dakota.

Some Pathogenic Fungi Occurring in the Seed of Red and Subterranean Clover.[1]—In attempts to obtain red clover (*Trifolium pratense* L.) seedlings free of fungi, it was found that surface-sterilized seed often gave rise to fungi of known pathogenicity for clover. It seemed desirable, therefore, to determine the presence and prevalence of these fungi in the seed of red clover.

Thirty-four lots of red clover seed less than 1 year old, 15 lots 2 or more years old, and 6 lots of subterranean clover seed were tested. These seed lots were surface-sterilized by immersion in 95 per cent alcohol for 1 minute, a 1–1000 aqueous solution of bichloride of mercury for 7 minutes, and a saturated solution of calcium hypochlorite until transferred to potato-dextrose-agar plates. Notes were taken 10 to 21 days later. A total of 28,161 red clover seeds and 3,549 subterranean clover seeds were plated. Of the 34 lots of red clover seed less than 1 year old, 19 were infected with *Pleospora herbarum* (Pers. ex Fr.) Rabh. (*Stemphylium botryosum* Wallr.), 6 with *Stemphylium sarcinaeforme* (Cav.) Wilts., 1 with *Cercospora zebrina* Pass., and 2 with a sterile black fungus of large mycelium. The latter fungus, first reported from Kentucky,[2] causes what is called the "black patch" disease of red and white clovers. This fungus has been reported also by Smith.[3]

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[1] Contribution No. 33 of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Northeastern States.

The prevalence of these fungi under conditions of the tests was not great, the highest in any lot being 2 per cent with respect to *Pleospora herbarum,* and less than 1 per cent with the other fungi. It was necessary to plate out 1,635 seeds of one lot to demonstrate the presence of the "black patch" fungus in 2 seeds. Only *Pleospora herbarum* and *Stemphylium sarcinaeforme* were demonstrated in viable seed. Other fungi isolated were species of *Alternaria, Phoma, Fusarium, Penicillium, Oospora,* and *Chaetomium.* No fungi were found in the 15 seed lots 2 or more years old.

TABLE 1.—*Blemished and blighted seed of 5 lots of red clover, separated from macroscopically sound seed, surface-sterilized, and plated on agar. (Note the much higher incidence of fungi in the blemished seed)*

Condition of seed	No. seeds tested	No. seeds infected	Number of seeds with		"Black patch" fungus	*Pleospora herbarum*	Other fungi
			Alternaria sp.	*Stemphylium sarcinaeforme*			
Sound	2,225	32	26	0	0	0	6
Blemished and blighted	2,450	313	271	6	6	1	40

Of the fungi shown in Table 1 *Pleospora herbarum*[4] and *Stemphylium sarcinaeforme*[5] have been reported previously from red clover seed. It is possible that the introduction of the "black patch" fungus into new fields occurs through the seed, as no spores of this fungus are known to occur.

Isolations were made also to determine what fungi were present in the seed of subterranean clover (*Trifolium subterraneum* L.). From one lot of 980 seeds, *Sclerotium bataticola* Taub. was isolated 4 times, *Rhizoctonia solani* Kuehn once, and *Fusarium* 5 times. No fungi were isolated from 5 other lots of seed. The results of this preliminary test are included here, since the fungi isolated are known to be pathogenic on other crops.—ST. JOHN P. CHILTON, formerly with the U. S. Regional Pasture Research Laboratory, State College, Pa.; now with the Department of Botany, Bacteriology, and Plant Pathology, Louisiana State University, Baton Rouge, La.

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[2] Forty-sixth Annual Report of the Kentucky Agricultural Experiment Station (1933): (1) 1–69. 1934.

[3] Smith, O. F. A leaf spot disease of red and white clovers. Jour. Agr. Res. [U.S.] 54: 591–599. 1937.

[4] Smith, O. F. *Stemphylium* leaf spot of red clover and alfalfa. Jour. Agr. Res. [U.S.] 61: 831–846. 1940.

[5] Krakover, L. J. The leaf-spot disease of red clover caused by *Macrosporium sarcinaeforme* Cav. Rept. Mich. Acad. Sci. 17: 273–328. 1917.

PRUNE DWARF

E. M. HILDEBRAND

(Accepted for publication January 17, 1942)

INTRODUCTION

Since the first announcement in 1936 (7), important information has been accumulated on the dwarf disease of prune (Prunus virus 6 (6); *Nanus pruni* H (5)). Originally found only in Niagara County, New York, the disease has since been observed in Canada. From the economic standpoint it is a minor disease, which, for some unknown reason, has become locally a serious problem in a few orchards. The standard control recommendation for the prompt removal of diseased trees has not eliminated the trouble, since in certain cases it has reappeared again within a few years. While studies aimed at solving this difficulty have not yet been concluded sufficient progress has been made during the past 6 years to deserve publication.

DAMSON PLUM MASKS PRUNE-DWARF VIRUS

The causal virus of prune dwarf may be even more widespread than is realized at present owing to the fact that the Damson plum has been found to harbor the virus without showing symptoms. The discovery that the prune-dwarf virus was harbored by Damson plum came about as the result of top-working a few Damson misplants to Italian prune in one of the commercial orchards under observation. In 1932 this orchard of about 230 trees had 2 widely separated diseased trees immediately adjacent to, respectively, 7 and 3 erect growing plums, identified as Damson, that had accidentally gotten into the orchard. When the cions that grew following grafting in 1933 all showed foliage symptoms of prune dwarf two possibilities were suggested: (1) that the grower had inadvertently used cions from diseased trees or (2) that the Damson plum trees harbored the causal virus.

All attempts to demonstrate the presence of the virus in the Damson plum trees in the orchard mentioned have given positive results. Cions and buds from Damson and from both healthy and diseased Italian prune were taken from this orchard and indexed on Italian prune trees at Ithaca in 1935, 1937, and again in 1939, employing a minimum of two trees for testing each source, with the same results each time. Transmission of the dwarf disease was always effected from the symptomless Damson trees and the diseased Italian prunes but never from symptomless trees of Italian prune.

VIRUS HARBORED IN NURSERY AND ORCHARD DAMSON PLUMS

To check on how widespread were virus carriers among Damson plums, trees were procured from several sources including nurseries and orchards, 3 from within and 2 from without New York State. Not all sources contained the virus but 1 out-of-state source and 2 from within New York gave positive results when indexed on Italian prune. How widespread this virus may be in plum varieties that mask symptoms remains to be determined, but, thus far, indications are that still other varieties may be involved.

In 1939 G. H. Berkeley and R. S. Willison showed the writer an interesting case near Grimsby, Ontario, where a grower had topworked Damson plums to Italian prunes about 3 years previously, with the result that a large percentage of the prune cions became infected. This observation confirmed the results of the New York experiments, and the evidence at hand rather conclusively demonstrates the fact that some of the Damson plums are masked carriers of prune-dwarf virus.

VIRUS SYMPTOMS MASKED BY BRADSHAW PLUM

That the Bradshaw plum might be a masked carrier of the prune-dwarf virus was indicated when symptoms failed to develop following bud inoculation of trees, but proof of this fact has only recently been established. In one Italian prune orchard from which all diseased trees had been previously eradicated a new outbreak occurred in 1936, followed by a further increase in the number of diseased trees in 1940. Careful examination revealed two Bradshaw (Niagara) plum trees amidst the newly infected ones. Dormant scions taken from the trees in question were grafted on two plants each of several varieties of plums including Italian (Fellenberg), Lombard, Damson, and Bradshaw. Symptoms developed within 6 weeks on Italian prune and Lombard plum. Whereas the Damson and Bradshaw varieties remained normal in appearance, it was subsequently demonstrated by indexing on peach seedlings, employing a rapid subsequently technique (4), that they were harboring the virus.

RATE AND MANNER OF SPREAD IN ORCHARDS

Evidence on rate and manner of spread of prune-dwarf virus between trees in orchards over a 10-year period may provide a clue as to the natural agent of dissemination. In the orchards under observation, which varied each year from 3 to 7 during the above period, the increase in number of diseased trees was intermittent. Although most of the orchards were removed before yielding data on this point, two exceptions will be cited. Orchard A contained 58 trees, approximately 25 per cent of which were diseased in 1932. The grower removed the defective ones and replanted to prunes. The disease reappeared on one tree in 1937. The number of diseased trees jumped to 6 in 1940, and, although they were hit and miss, they were confined to the same end of the orchard. Orchard B, about 230 trees, showed 2 diseased trees in 1932, 11 in 1937, 13 in 1938, and 16 in 1940, when the entire orchard was removed. The new infections all appeared immediately adjacent to the two initial infections. Both orchards had severe infestations with the green plum aphis in 1936 and lighter infestations in 1939.

The possibility of spread by the pruning operation seems remote, since the common mechanical transmission methods that have been tested have always failed to give positive results.

The above circumstantial evidence that the green plum aphis may be the vector of prune dwarf has not been borne out thus far by caging experiments with this insect (7). However, after concluding the above mentioned caging experiments, one of the 4 prune trees developed symptoms in 1935. The fact that this tree was in the open and exposed to environmental factors beyond control makes the evidence merely suggestive, not conclusive. Besides, some other agent than the aphid may have been involved.

The tendency for this disease to spread to the immediately adjacent healthy trees, with occasional skips, suggests a short flight range of the natural vector, a possibility that fits in rather well with the suspicion that an aphid may be involved. The studies on insect transmission, conducted both in the greenhouse and outdoors, that have been in progress for several years have not been concluded.

EFFECT ON YIELDS

The striking effect of this disease on fruitfulness can be gleaned from actual yields of young prune and plum trees shortly after they came into bearing. Two adjacent 6-year-old Italian prune trees, one diseased and the other healthy, yielded as follows: (1) total number of fruits, 7 and 115; total weight of fruit, 8 and 101 ounces; average weight of fruit, 1.14 and 0.88 ounces. The yield from the diseased tree was less than 8 per cent of normal and closely approximates the estimates on yields of old orchard trees, which have ranged between 5 and 10 per cent of normal over a period of years. Whereas the prune type of plum has two striking and distinctive symptoms of dwarfing and narrowing of leaves and almost complete loss of fruitfulness due to abortion of pistils, the effect on Lombard plum (Fig. 1) seems to be largely confined to leaf symptoms. The fruit yield of a healthy Lombard plum tree compared with the average yield of 3 adjacent diseased trees was as follows: (1) number of fruits, 115 and 175; (2) total weight of fruits, 85 and 97 oz.; (3) average weight of fruit, 0.74 and 0.55 oz. The smaller size of fruit on the diseased trees was attributed to the larger number of fruits and to the reduced leaf surface. The fruit yields of symptomless Damson plum trees that carried the virus seemed not to be affected at all.

TRANSMISSION EXPERIMENTS

Although the original experiments (7) indicated that only plums were susceptible to infection by the prune-dwarf virus, additional experiments have been conducted on plums, cherries, and peaches.

The customary inoculation procedure was to place a diseased bud or cion at least midway up the stem of a young 1- or 2-year-old plant, and, where 2 buds or cions were used, at least one was placed high on the stem.

Plum to Plum

The only essential for successful transmission from prune to prune was tissue union between cion and stock for a relatively short interval of a few weeks. Several types of graft (bud, cleft, whip) employed in 8 separate experiments, involving 5 or more trees each, all proved equally effective and 100 per cent efficient in transmission experiments with the prune. The details of these experiments have been omitted to save space. It was found that placing the grafts midway or higher up on the stem hastened the appearance of symptoms. The incubation period varied from about 5 weeks

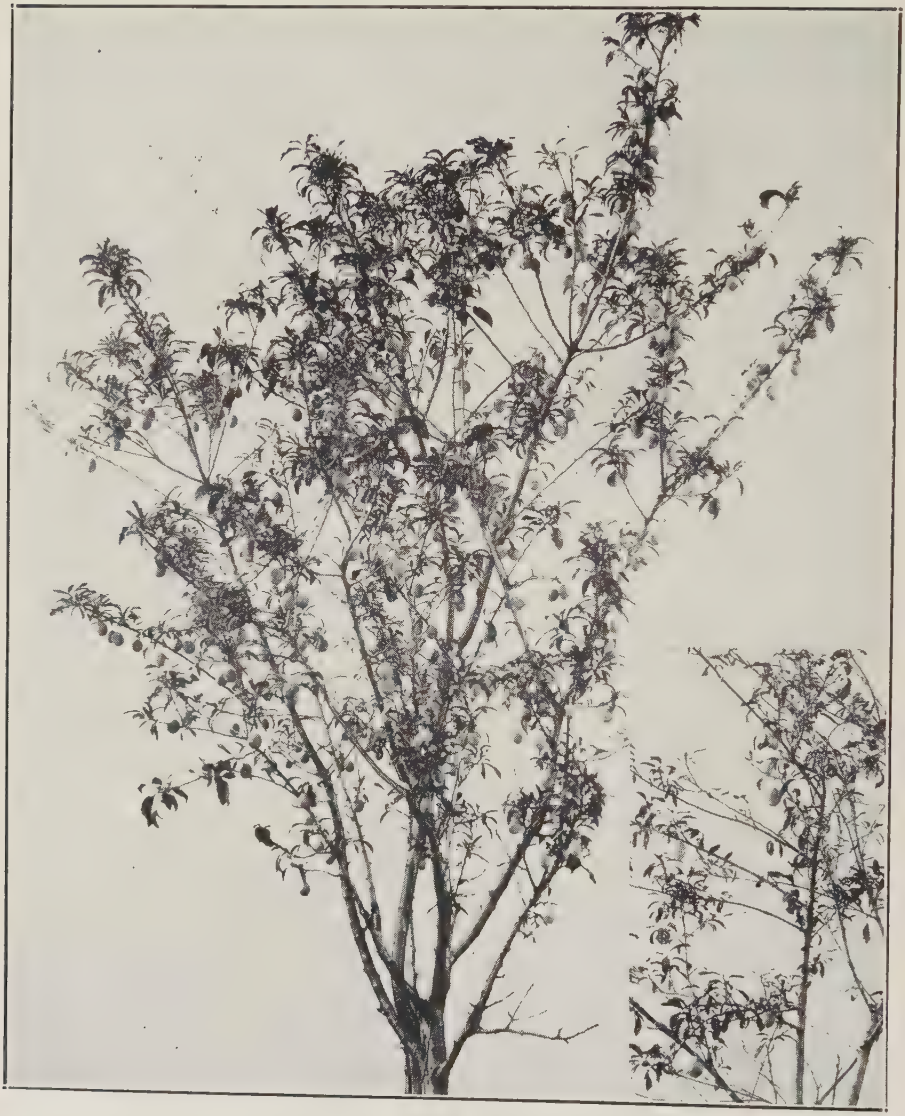

FIG. 1. Lombard plum tree, severely affected with prune dwarf, on which the foliage is dwarfed but the fruit crop apparently normal. The inset shows portion of Italian prune tree with characteristic dwarfing of foliage and sparsity of fruit.

to a year or longer, depending on when during the year the inoculations were made, the position of the grafts on the stems, and the growth condition of the plants employed.

The first symptoms to appear on prune were chlorotic rings (Fig. 2) on otherwise normal appearing leaves in close proximity to the position of the diseased tissue about 5 weeks after grafting. As the season advanced this symptom had a tendency to become less conspicuous, whereas the later-formed leaves in the tip region became markedly dwarfed. Figure 2 illustrates typical leaf symptoms on a plant in September, which had been budded at the beginning of the growing season, ranging from chlorotic rings near the base to the dwarfed narrow leaves at the tip of a shoot.

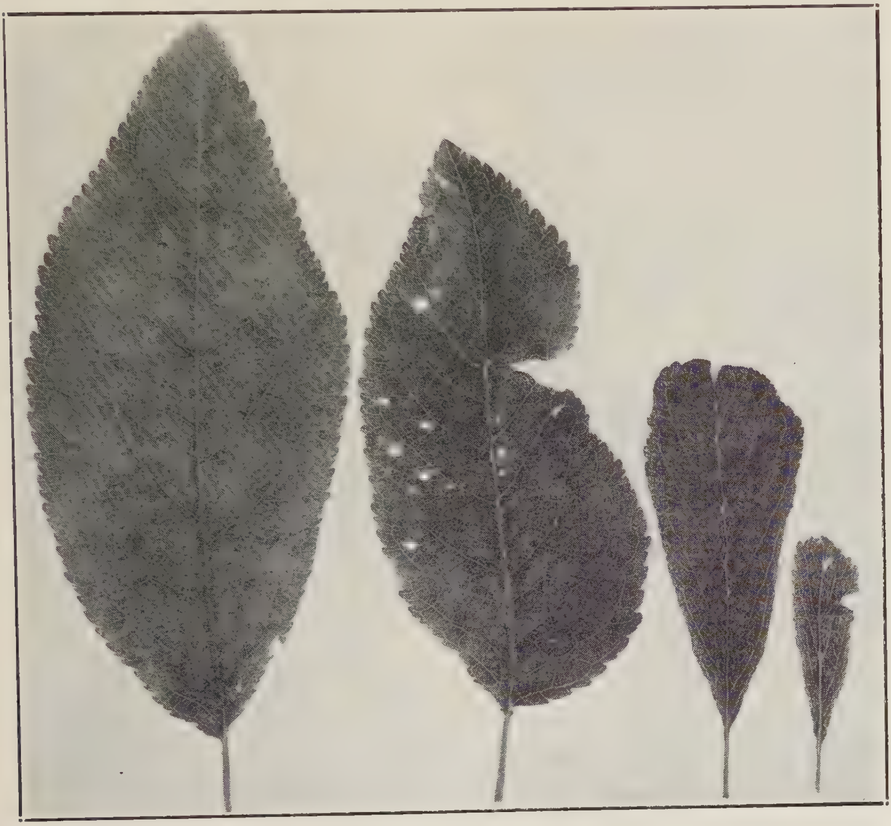

FIG. 2. Symptoms of dwarf on current-season foliage of Italian prune—ring spot, shot hole, malformation, and dwarfing—representing the range in expression from the base to the tip of a single shoot of a tree, in September, that had received a diseased bud early in the spring.

Transmission experiments conducted between Italian prune and several plum varieties: (*Prunus domestica*—Italian (Fellenberg), Smith, German, Lombard, Bradshaw and Reine Claude; *P. salicina*—Abundance, Burbank, and Red June; *P. insititia*—Damson (Shropshire)) gave interesting results. Severe symptoms including severe dwarfing of foliage and light fruit set were always produced on the prune types of plums—Italian, Smith (? Italian), German. The Lombard plum had moderately severe foliage symptoms but apparently without injury to fruit set. On the two remaining *P. domestica* varieties—Bradshaw and Reine Claude—significant symptoms were not produced, although there appeared to be a slight reduction in size of foliage. Unfortunately, the planting containing these two varieties was lost before determining whether they were carrying the virus.

The results of the transmission tests between the prune and the *P. salicina* varieties of plums were apparently negative except for a slight dwarfing of foliage but difficult to diagnose because of the presence of other viruses.

In each of 3 separate nursery shipments of dormant trees of the Red June variety of plum one or more trees were found carrying a line-pattern virosis (2) when grown in the greenhouse. It was noted that the trees showing the chlorotic line patterns bore a lighter green foliage. Ordinarily, they showed the striking line pattern symptoms at the lower temperatures of spring, becoming masked with increase in temperature as the season advanced. The affected trees were otherwise apparently normal.

Occasional trees of the Abundance and Burbank varieties also showed a lighter green color of foliage and, since this abnormality also occurred in the checks, it could not be attributed to the prune-dwarf virus, also being presumably a contamination originating in the nursery. Incidentally, one Abundance tree had what was considered a typical line pattern, much fainter in outline than that seen on Red June. Unfortunately, for lack of space, these experiments were terminated without determining whether or not the *Prunus salicina* varieties were carrying the prune-dwarf virus.

As already mentioned, Damson plums (*P. insititia*) from several sources were found to be masked carriers of the prune-dwarf virus. The lesson to be drawn from the above transmission experiments on plum clearly indicates the importance of growing, observing, and indexing all test plants on other *Prunus* spp. before using them in host-range studies. The recent report from Canada (1) cites two good cases at point. It is further indicated that failure to show symptoms is not a reliable criterion for absence of virus.

Plum to Cherry

The results of transmission tests from plum to cherry have apparently all been negative. Two sour-cherry varieties (Early Richmond and Montmorency) and 4 sweet cherries (Black Tartarian, Napoleon, Yellow Spanish, and Windsor) were employed. For each variety 3 plants received 2 buds each from diseased prune, 1 plant 2 buds from healthy prune, and the fifth plant was not budded. At the end of 2 years no symptoms were visible on these plants, with one exception. A single shoot on one of the Black Tartarian trees showed a marked dwarfing of leaves and stunting of growth. The growth was so short that a bud could not be taken for transmission purposes.

The identity of a chlorotic ring-spot and line-pattern disease, which appeared on a Mahaleb root sprout from one of the Montmorency trees, was not determined because it failed to produce symptoms when budded into peach seedlings. This latter disease was observed again in 1941 in a cherry nursery on Mahaleb suckers coming up from roots where sour-cherry buds failed to take the previous season. Whether or not it was responsible for these failures is not known.

Plum to Peach

Transmission to healthy Elberta peach trees was effected by grafting from diseased prune in 5 separate experiments, all but one of which were conducted in the greenhouse. The outdoor experiment was conducted in the writer's garden under good growing conditions. Therefore, failure to transmit this disease in earlier attempts (7) was very probably due to poor growing conditions rather than immunity on the part of the plant. Since the results from these experiments were very similar, only two will be discussed here, the third and fourth, conducted, respectively, in the greenhouse and outdoors.

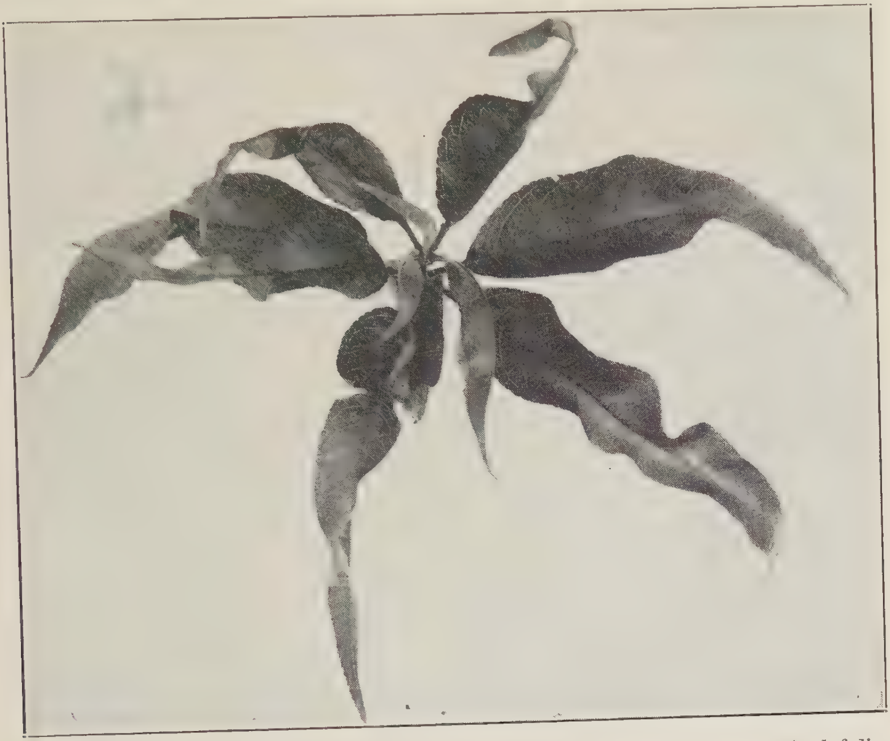

FIG. 3. Early-stage symptoms of prune dwarf on newly developed terminal foliage of Elberta peach, consisting of leaf distortion, wavy margins, and a chlorosis of the distal half, where deformation was most marked. This tree was budded in September, then placed outdoors till mid-January. The photograph was taken in February, about 1 month after the trees had been returned to the greenhouse (65° F.) for forcing.

The third experiment, begun on August 31, 1936, employed 39 2-year-old

Elberta peach trees growing in pots, 10 receiving 2 buds each and 19 one bud each, the remaining 10 being held as checks. To verify the presence of prune-dwarf virus in the budwood 4 Italian prune trees also were included, each receiving 2 buds. After budding, the trees were placed outdoors. They became dormant about November 1, without showing any symptoms.

The first symptoms suggesting transmission of the dwarf disease to peach were noted on February 17, 1937, or about a month after the trees had been moved from outdoors to the 65° F. greenhouse on January 15, and consisted mainly of leaf distortion (Fig. 3). Ten days later occasional chlorotic spots (Fig. 4) appeared on several but not all of the leaves. The distortion shown in the figure was largely confined to the distal halves of the younger leaves, which became narrower, wavy margined and lighter green, whereas the chlorotic spots appeared more commonly on the older more nearly normal shaped leaves. Ten out of the 19 trees receiving one diseased bud and 9 out of the 10 trees receiving two buds showed these symptoms on February 27. As the season advanced the greenhouse temperature was raised to 70°

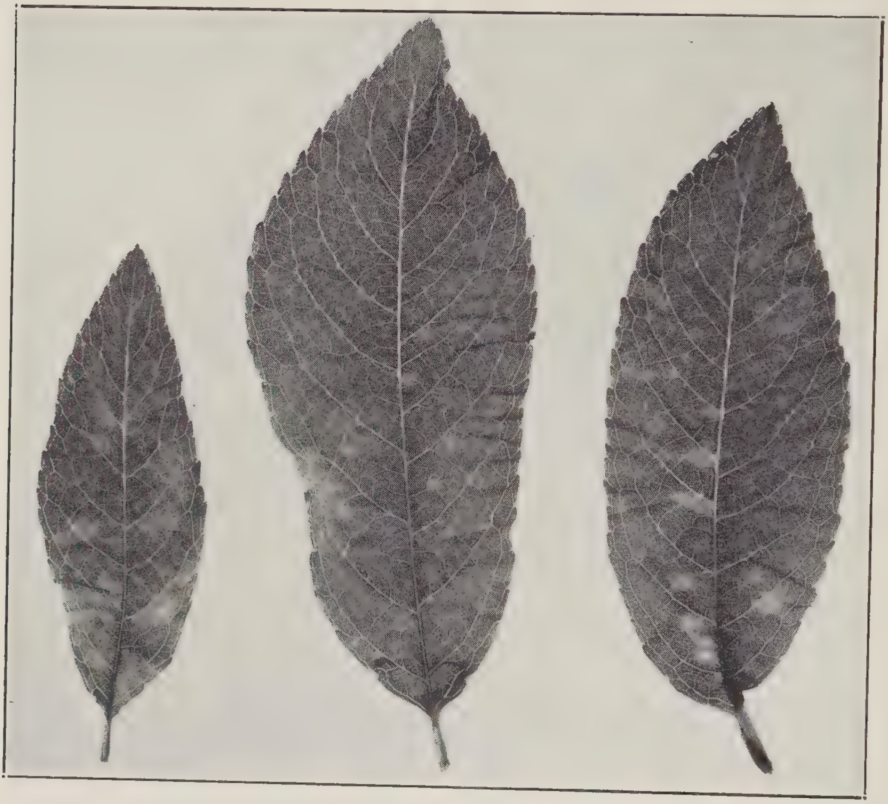

FIG. 4. Symptoms of prune dwarf on Elberta peach, which followed about 10 days after the stage shown in figure 3. The chief characteristics were chlorotic spots, mild crinkling and shortening of the leaf blade, the effect being most pronounced in the basal half of the older leaves.

F. and above. With the rise in temperature the affected leaves gradually recovered from the two types of chlorosis and from much of the distortion, except the waviness of the leaf margin. The next development, especially on the plants receiving 2 diseased buds, was a stunting of shoot growth accompanied by a variable amount of leaf drop and marginal leaf spot on the older leaves, and a tendency for the shoots to develop rosette similar to

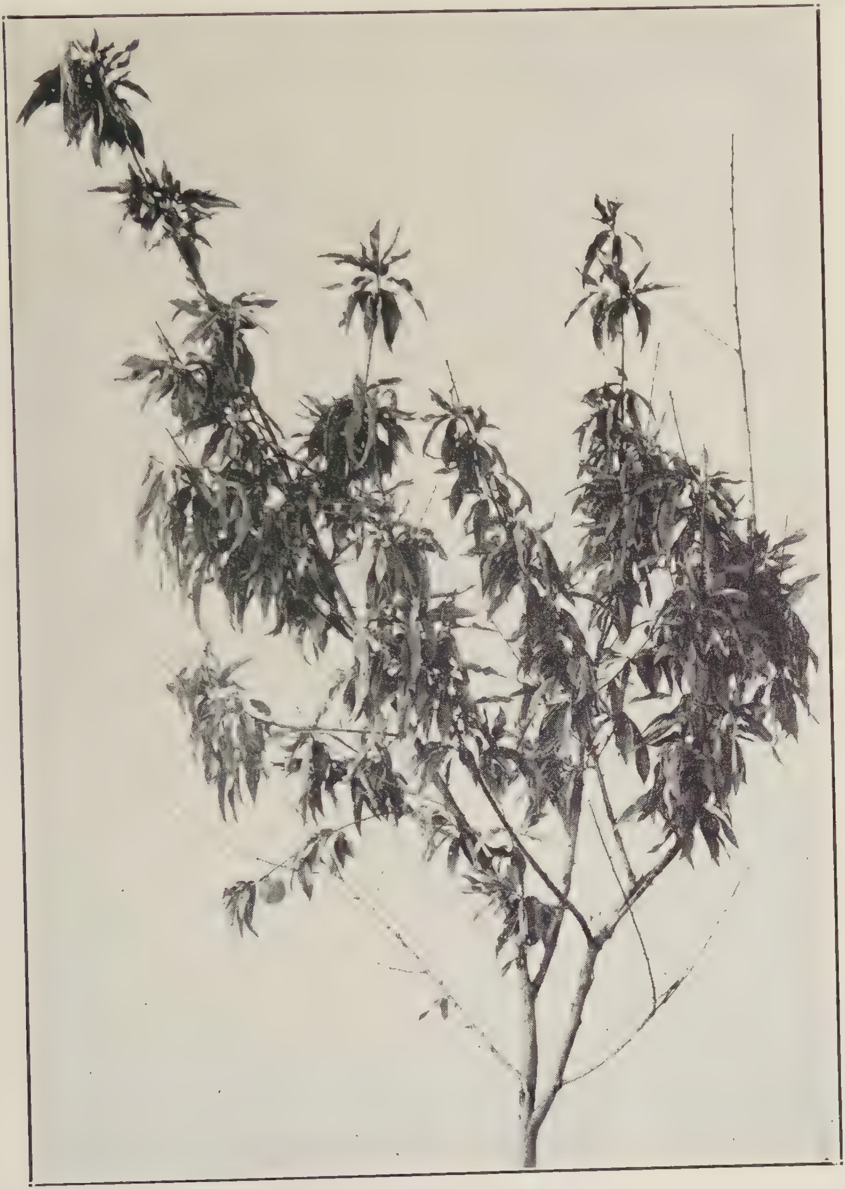

FIG. 5. Prune dwarf on peach showing typical rosetting and wavy leaf on a 4-year-old Rochester peach tree grown in fertile garden soil. The tree was planted as a 2-year-old in 1935, received four diseased buds from prune in 1936, and was photographed in 1937. The dead twigs were the result presumably of winter injury.

but milder than that induced by rosette-mosaic on peach (3). Later, with the coming of summer temperatures, the majority of the affected plants had largely recovered from the more conspicuous of the symptoms. A year later these trees appeared to have almost completely recovered from the disease, except possibly for a slight waviness of leaves and stunting. On March 20 the 4 check prune trees were showing typical early-stage symptoms ranging from chlorotic ring spots of the older leaves to willowing of the tip leaves on the terminals.

An interesting angle of this experiment was the fact that nearly all of the peach trees, even though only beginning their third season of growth, blossomed, and a moderate number bore fruit to maturity, thus affording an opportunity to study fruit symptoms. Five of the diseased trees matured fruit in June. In every case the suture side developed slower than other parts of the fruit, was slightly rough and off shape, and, instead of yellow with a red blush, as in the checks, was greenish-yellow, which contrasted with the prevailing naphthalene-yellow over the remainder of the fruit. The fruits on affected plants were smaller and matured from 7 to 15 days later than those on the check plants.

The outdoor experiment was conducted on 2 trees each of 3 peach varieties—Elberta, Rochester, and South Haven. These trees were planted when 2 years old on May 2, 1935, and were making excellent growth when 1 tree of each variety was grafted with 4 diseased buds on August 6, 1936. All 3 budded trees were showing symptoms in 1937, the most distinctive being a stunting of the terminal growth, simulating rosette and the tendency of the leaves to have wavy margins (Fig. 6). It will be remembered that these same symptoms were the end result of the greenhouse experiment just described. The following year these trees likwise seemed to have recovered from the disease.

RESEMBLANCE TO ROSETTE-MOSAIC DISEASE

Because these symptoms, although milder, bore a resemblance to the rosette-mosaic disease on peach, comparative studies were undertaken with a view to clarifying their relationship (3). Although this study has not been concluded, the results to date suggest that if a relationship exists the virus causing rosette mosaic is a much more aggressive strain on the peach than is prune dwarf, whereas the latter virus is most aggressive on the prune. The recent unreported finding by Cation in Michigan of a mild strain of rosette-mosaic virus may have a bearing on this point since it suggests the existence of strains within that virus.

SUMMARY AND CONCLUSIONS

Originally found only in Niagara County, New York, prune dwarf is now known to be present also in Canada.

The Damson plum is known to harbor the prune-dwarf virus without showing symptoms, and the causal virus may be more widespread than is realized because of this fact. Three out of five nursery and orchard sources of Damson plums were positive for the virus when indexed on Italian prune.

Two severe infestations of Damson plum with this virus have been reported in Canada, where the growers top-worked to the susceptible Italian prune. Symptoms of prune dwarf are also masked by Bradshaw plum.

The disease spreads slowly and, ordinarily, to the immediately adjacent trees in orchards but in spurts which have been correlated with severe infestations with the green plum aphid. Insect transmission experiments, however, have all been negative thus far.

Fruit yields on the prune types of plums are extremely reduced, averaging less than 10 per cent of normal. The Lombard plum, which suffers foliage symptoms but not the abortion of pistils, yielded slightly below normal, apparently because of the reduced foliage. Fruiting of the Damson plum, which masked symptoms, seemed to be normal.

The only essential for successful transmission to susceptible plants was tissue union between cion and stock. The shortest incubation period for prune was 5 weeks. Of the *Prunus domestica* varieties tested, only Lombard plum, besides the prune types, developed typical foliage symptoms. None of the *P. salicina* varieties developed marked symptoms, but it was found that a distinct chlorotic line pattern virus was prevalent in Red June and also present in Abundance. The Damson plum (*P. insititia*), while failing to develop typical foliage symptoms, was, subsequent to budding, found to contain the virus. Transmission tests from prune to cherry have all been negative. The peach was found susceptible to prune-dwarf virus with the production of a number of foliage symptoms from which the plants largely recovered as the season advanced. The peach fruit on affected plants was retarded in development, especially on the suture side. The symptoms that appeared in both outdoor and greenhouse tests with Elberta peaches were a stunting in terminal growth, simulating a mild case of rosette and the tendency toward wavy margins for some of the leaves. By the following year the affected peaches had largely recovered from the disease.

Studies are under way to determine what relation, if any, exists between prune dwarf and peach-rosette mosaic, to which it bears a definite resemblance in symptoms on peach.

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A ROOT ROT OF COTTON CAUSED BY THIELAVIOPSIS BASICOLA

C. J. King and J. T. Presley[1]

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INTRODUCTION

In 1922 a peculiar root rot of cotton plants was observed at the U. S. Field Station, Sacaton, Arizona. Affected plants were characterized by main roots, swollen at the collar and manifesting an internal purplish-black discoloration. It was first thought to be a disease that appears on mature plants in late summer or early fall, and was described as an internal collar rot (3). Later, it was observed in the spring destroying seedlings 3 to 4 weeks old. During the hottest part of the season it is not evident. It reappears with the approach of fall, when further mortality of cotton plants occurs (Fig. 1, A). It was noted early that the disease affected American-Egyptian cotton more severely than it did the upland type. It also showed great persistence in poorly drained soils, and recurred in cotton after several years of rotation with alfalfa and other nonsusceptible crops. In 1940 it was found in a commercial field of American-Egyptian cotton in the Upper Gila River Valley, nearly 200 miles east of Sacaton, but in the same drainage area and soil type. In 1938 the causal organism was identified as the fungus *Thielaviopsis basicola* (Berk. and Br.) Ferraris, cause of black root rot of tobacco in Europe and in some of the eastern and mid-western States of the United States.

For many years the fungus deemed responsible for black root rot of tobacco carried the name *Thielavia basicola* Zopf. The work of McCormick (5) in 1925 furnished evidence that *Thielaviopsis basicola,* which usually develops two spore forms, endoconidia and club-shape chlamydospores, is the actual cause of black rot. An ascogenous fungus, frequently associated with it, and long believed to be a form of the causal organism, was given the designation *Thielavia basicola.* The latter is not known to be parasitic. In cultures it has a tendency to produce perithecia, especially if grown in the presence of *Thielaviopsis* or certain other fungi. *Thielavia* has not been observed in association with *Thielaviopsis* on cotton in Arizona.

The report in 1939 of an internal collar rot of cotton in Arizona, caused by *Thielaviopsis basicola* by King and Barker (4), apparently is the first record of this fungus in the arid Southwest, and also of its damage to cotton under field conditions. Smith (9), Rosenbaum (7), and Johnson (2) previously had reported it as causing a root rot of cotton under controlled experimentation; and, in 1940, Sherbakoff (8) reported it as responsible for injuries to cotton seedlings at Knoxville, Tennessee. The disease is not

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[1] The writers gratefully acknowledge the assistance of Lloyd A. Brinkerhoff in laboratory and greenhouse work connected with this investigation while employed as Agent at the U. S. Field Station, Sacaton, Arizona, December 1, 1939, to April 12, 1940, and to H. D. Barker for many helpful suggestions.

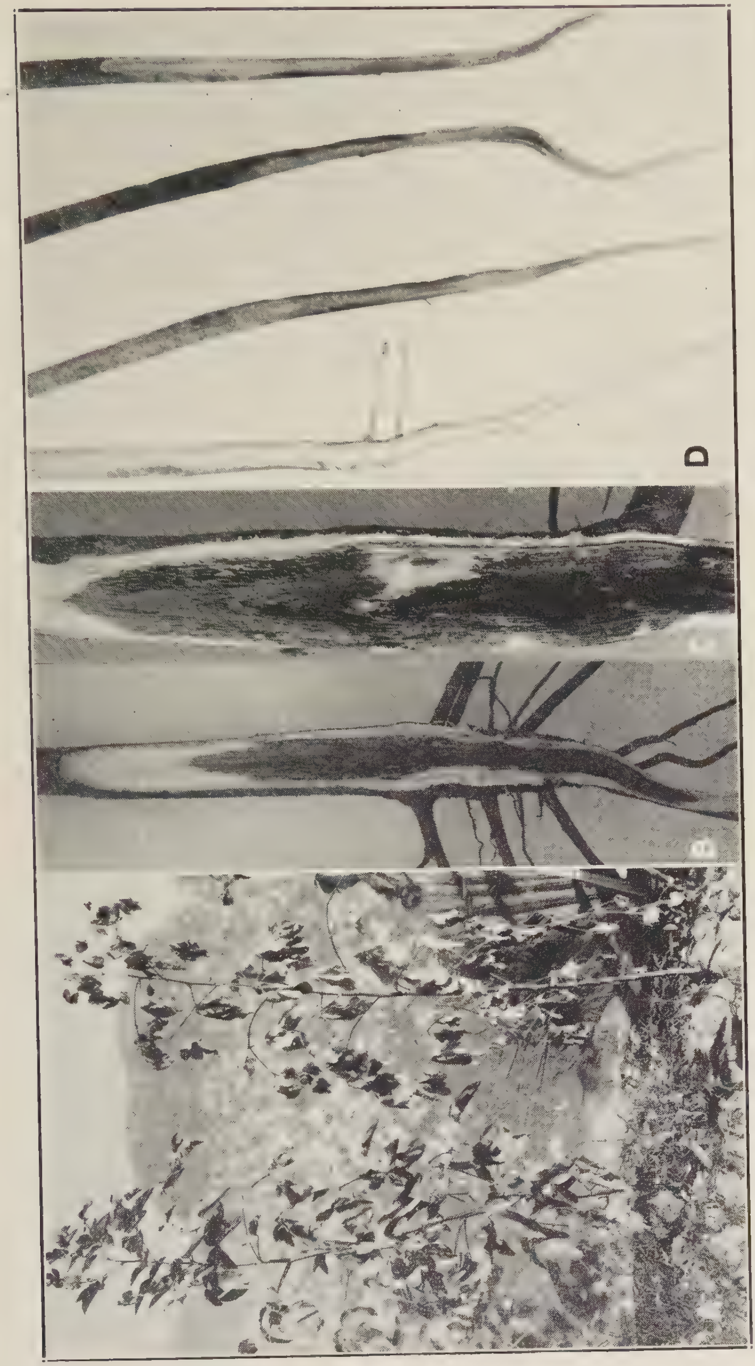

FIG. 1. Symptoms of Thielaviopsis root rot on Pima (American-Egyptian) cotton plants. A. Plant at right dying late in the season; plant at left not affected. B. and C. Internal collar rot symptoms. Note swelling at the crowns. D. Three taproots at right show typical injuries to seedlings about 6 weeks old. Root at left not affected.

believed to be of common occurrence in Arizona, but its discovery in two widely remote locations on the Gila River suggests that it may be indigenous.

HISTORY

After discovery of internal collar rot of cotton at Sacaton in 1922, specimens of affected roots were sent to several pathological laboratories, but no parasitic organisms were isolated. In 1924 G. L. Peltier, while temporarily employed in Arizona by the Bureau of Plant Industry, examined some affected plants and prepared a preliminary description of the disease, which remained unpublished pending results of some culture work he was unable to complete before resuming his regular duties in Nebraska. In later years several unsuccessful attempts were made at Sacaton to isolate the causal organism. In January, 1938, it was observed that the discolored root tissues, particularly the conducting vessels, invariably showed numerous dark colored chlamydospores. This discovery aided in the selection of recently invaded tissues and the isolation of the organism, which formed the dark-brown chlamydospores. Inoculation tests proved that this was the causal organism.

Cultures of the fungus were submitted to the Division of Mycology and Disease Survey, Bureau of Plant Industry, and were identified tentatively by J. A. Stevenson as *Thielaviopsis basicola.* For comparison, type cultures of *Thielaviopsis basicola* were obtained from the tobacco experiment station at Greenville, Tennessee, and from Washington University, St. Louis, Missouri. Comparative cultural and inoculation studies indicated that the Arizona organism and the tobacco isolates belonged to the same species, but that they differed slightly in cultural characteristics.

SYMPTOMS AND EFFECTS

When the disease is studied on cotton plants in the seedling stage, the aboveground symptoms somewhat resemble the effects of root-knot nematodes or injuries from toxic salts: The seedlings are stunted and apparently unhealthy; the leaves are often small and cupped, pale green, purplish along the veins, and show marginal browning and burning. Gradually the lower leaves wither and drop off; many seedlings succumb at various stages between the development of the 3rd and 6th leaves.

The roots of the affected seedlings show a purplish-black discoloration of the affected tissues (Fig. 1, D). As in the case of mature plants, the central cylinder usually shows the first effects of invasion by the fungus. When the main root of a seedling with mild symptoms is split, it is observed that the stele already is discolored and often portions have disintegrated to form cavities. In plants with more severe symptoms the roots show portions of the cortex in a black, shrunken condition. By the time the plants are dead nearly all of the cortex, as well as the stele, is involved, and the entire root is so fragile that little remains with the plant when it is pulled from the ground. As temperatures rise with the advance of summer, the affected young plants that have survived begin to recover; by mid-summer there is little evidence of the disease. On cutting through one of the recovered plants at the collar, a cylinder of necrotic tissue surrounded by healthy tissues is usually found. When lower temperatures prevail in late summer the infection extends from these occluded lesions and effects further damage to the plant, often causing its death.

The symptoms shown by mature plants when they wilt and finally die might easily be mistaken for Phymatotrichum root rot (Fig. 1, A). It is to be noted, however, that the leaves remain on the dead plants longer than in the case of death from most of the cotton diseases. Also, it is characteristic of occasional mature plants to die. It appears probable that many of these may be plants in which there is renewed activity of the organism in old lesions, although some of them may represent new infection that has developed after temperatures again become favorable. It is seldom that more than 2 or 3 plants die in one group.

In mature plants the first sign of the disease is an abnormal swelling of the main root, beginning at the collar and extending 3 to 6 inches below the ground line (Fig. 1, B). Usually, the bark tissue on the tap root appears healthy, except that the lenticels often are distended and occur as cork-like protuberances. Splitting the tap root reveals a highly colored, brown to purplish-black, heart rot affecting the entire stele of the swollen area (Fig. 1, C). The discolored tissues for the most part are firm, with only a few small cavities to indicate a slow, dry-rotting process. The line of demarkation between the white healthy tissues and the discolored, diseased tissues is very distinct. The discoloration seldom extends far above the ground level. Immediately after the death of the plant, the only evidence of external decomposition is noted on the ends of the lateral roots and far down on the tap root. Here the entire root is involved and the bark may slough off on handling. Later, the cortex of the swollen area on the main root is broken through and black sunken areas appear on the outside. A general decay then occurs from attack of secondary organisms.

On diseased seedlings and occasionally on mature plants, the lateral roots may be finer and more numerous than on normal plants. This behavior is not so pronounced as that described for the tobacco plant.

THE ORGANISM

It was found that a microscopic examination of thin sections of discolored tissues provides an easy and reliable identification of the disease. Tissues, discolored by *Thielaviopsis*, invariably contain many brownish, multisegmented chlamydospores (Fig. 2, A). Chlamydospores abound in the conducting vessels and in cavities and intracellular spaces of the phloem, xylem, and medullary-ray structures. Occasionally, they may be seen within parenchyma cells. Endogenous spores rarely are seen within the host tissues. In their restricted positions within the tissues the chlamydospores do not attain the typical club shape, the regularity in number of

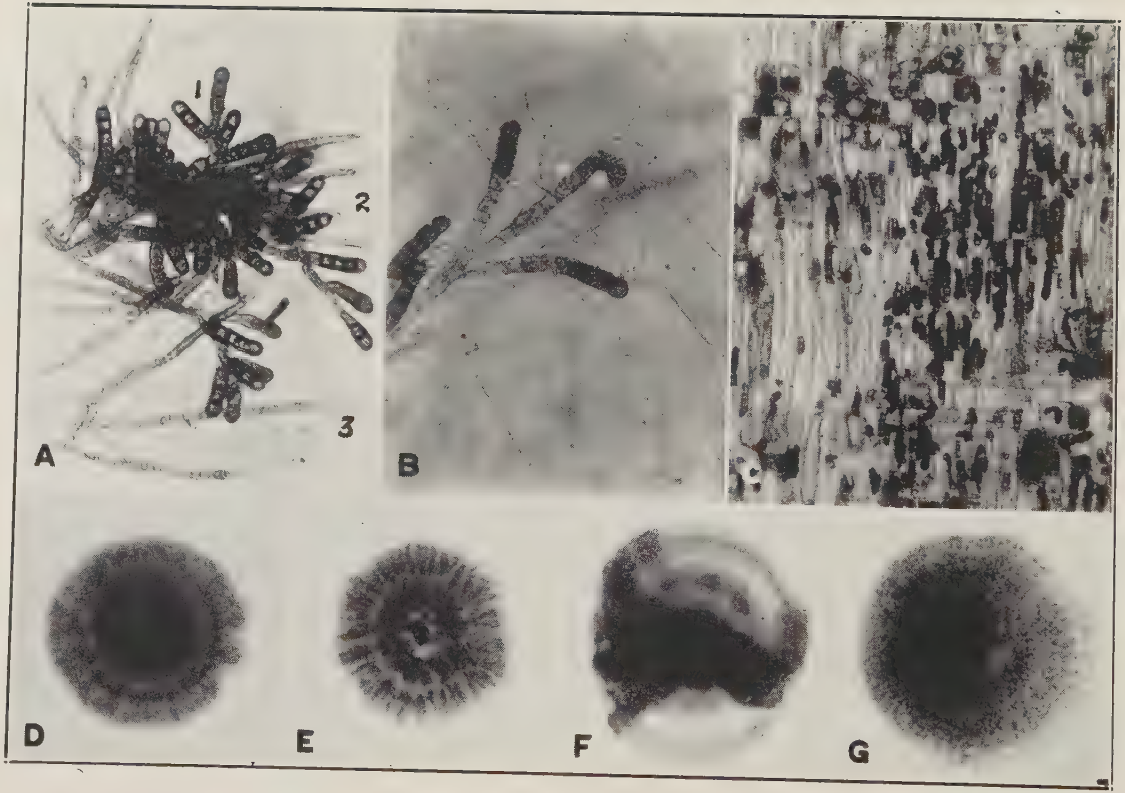

FIG. 2. Some characteristics of *Thielaviopsis basicola.* A. Types of spores produced in artificial culture: 1, Chlamydospores; 2, endospores; 3, endoconidiophore. B. Chlamydospores germinating. ×approximately 600. C. Chlamydospores within the vascular tissues—(longitudinal section of Pima root crown). D and F. Colonies of the cotton isolate (Arizona): D, 9-day-old culture on potato-dextrose agar; F, 12-day-old culture on onion agar. E and G. Colonies of the tobacco isolate (Missouri): E, 9-day-old culture on potato-dextrose agar; G, 12-day-old culture on onion agar.

segments, and the racemose arrangement on the hyphae, as is common on artificial media (Fig. 2, C). Usually they are cylindrical and consist of from 3 to 14 segments extending in chain-like formation through the vessels.

Cultural Characteristics

The Arizona Thielaviopsis fungus is readily isolated from cotton plants affected with black root rot, and can be grown on a wide range of culture media. On agars containing malt extract, potato dextrose, or decoctions of onion or of *Nicotiana glauca* the growth is vigorous, with endoconidia forming abundantly within 2 days and chlamydospores within 4 days after incubation at room temperature. On potato-dextrose agar the initial growth of the colonies is ashy-gray. Except at the margins, it soon becomes a blackish-brown imparted by the chlamydospores.

Comparisons were made of the growths of the Arizona cotton isolate and the tobacco isolate from Missouri grown on different media and at different temperatures. When grown on the same plates or in parallel series of plates on potato dextrose agars and incubated at 16, 21, 26, and 30 degrees C. the growth rates were about equal for both isolates, with most rapid growth occurring at 30° C. The average daily growth for both isolates at this temperature for the first 8 days after inoculation was 3 mm. Gilbert (1) reports 30° C. as the optimum temperature for growth of *Thielaviopsis basicola.* Rawlings (6), however, considered the optimum for 3 races of the fungus in his study as near as 20° C.

While the two isolates reacted much alike at different temperatures, they were easily distinguishable in appearance (Fig. 2, D and E). On potato-dextrose agar the colonies of the Arizona cotton isolate were lighter in color, denser, and more submerged than those of the tobacco isolate. Concentric zonation was pronounced in both, but more conspicuous in that from tobacco. No sectoring occurred on any of the cultures on potato dextrose. Johnson and Valleau (3), however, observed instability and sectoring in all of 11 tobacco isolates grown on potato-dextrose agar. On onion agar both the Arizona and Missouri cultures grew vigorously, but the mycelium of the cotton isolate when several days old was darker and more appressed (Fig. 2, F and G). Sectoring was a common tendency with the cotton isolate on this medium, but did not occur in that from tobacco. Transfers were made from one of the white or albino sectors and one of cinnamon-buff color. Both maintained these characteristics during several transfers on sweet-potato plugs and potato-dextrose agar. It seems significant that Rawlings (6) observed a tendency for his Texas isolate from cotton to develop sectors, particularly on onion agar, while an isolate from tobacco in Tennessee and one from *Primula obconica* in Holland failed to develop sectors on potato-dextrose, onion, or Leonian's agar.

Measurements of the endospores and chlamydospores of the two isolates showed no significant differences in size. The mean length of the endospores was approximately 14.5 μ and mean width 4.4 μ. The chlamydospores averaged 38 μ in length and 11 μ in width.

One noticeable difference was in the coloring of the hyphae bearing the chlamydospores. In the tobacco isolate the spore-bearing branches and basal cells of the chlamydospores usually were hyaline; whereas the brownish coloration of the isolate from cotton often extended into these structures.

Some germination tests were made with the endospores and chlamydospores of the cotton isolate of the Thielaviopsis fungus. In hanging drops of sterile tap water or on cubes of potato-dextrose agar a large proportion of the endospores germinated within 16 to 20 hours. The chlamydospores, however, failed to germinate under these conditions. When planted on dilute drops of malt agar containing a trace of pepsin, about 40 per cent of the chlamydospores developed germ tubes from one or more segments (Fig. 2, B).

INFECTION EXPERIMENTS

In 1939, inoculations with pure cultures of *Thielaviopsis basicola* were made on a small number of cotton plants in the field and greenhouse at 4 different times of the year. The varieties represented were Pima, Sea Island, and Sakellaridis of the Barbadense species and Acala of the Hirsutum species. In all cases the plants had reached the fruiting stage when inoculated. Longitudinal incisions with a sterile scalpel were made on the tap root just below the ground level. Tufts of spore-bearing mycelium were inserted and the incision was closed with adhesive tape. Half as many control plants received similar treatment, except for insertion of inoculum. The first examinations were made about 20 days after inoculation. With few exceptions the inoculated plants showed discoloration in the vascular tissues extending about 7 cm. from the point of inoculation on varieties of the Barbadense species, and about 4 cm. on varieties of Hirsutum. After 20 to 30 days chlamydospores were always observed within the discolored tissues, and a swelling of the tap root near the crown was often noticeable on plants of the Barbadense species but not on those of the Hirsutum species. A few of the plants were dwarfed and apparently unhealthy, but none of them died. The incision on the control plants healed rapidly, and discoloration failed to develop in the vascular cylinder.

In December, 1939, more elaborate greenhouse inoculation experiments were undertaken for comparing the pathogenicity of the Arizona cotton isolate and the tobacco strain from Missouri. Pima cotton and Maryland broadleaf tobacco plants were chosen for hosts. The latter were grown in crocks filled with clay loam. Some of the cotton was grown in soil, and some in sand nutrient cultures. The inoculum, consisting of pure cultures grown on sterilized cotton roots, was inserted in holes made in the soil or sand between the plants when the seedlings were only 2 weeks old. Within 9 days after inoculations were made on December 27, the tobacco seedlings began dying in all of the containers inoculated with either the cotton or the tobacco isolate, whereas the controls remained healthy. During the following 2 weeks more than one-third of the inoculated tobacco seedlings died from black root rot. Invariably, chlamydospores could be found in the diseased tissues. No further mortality of tobacco plants occurred; but, when final examination was made on March 25, all plants in inoculated containers showed damage from black root rot (Fig. 3, D–F).

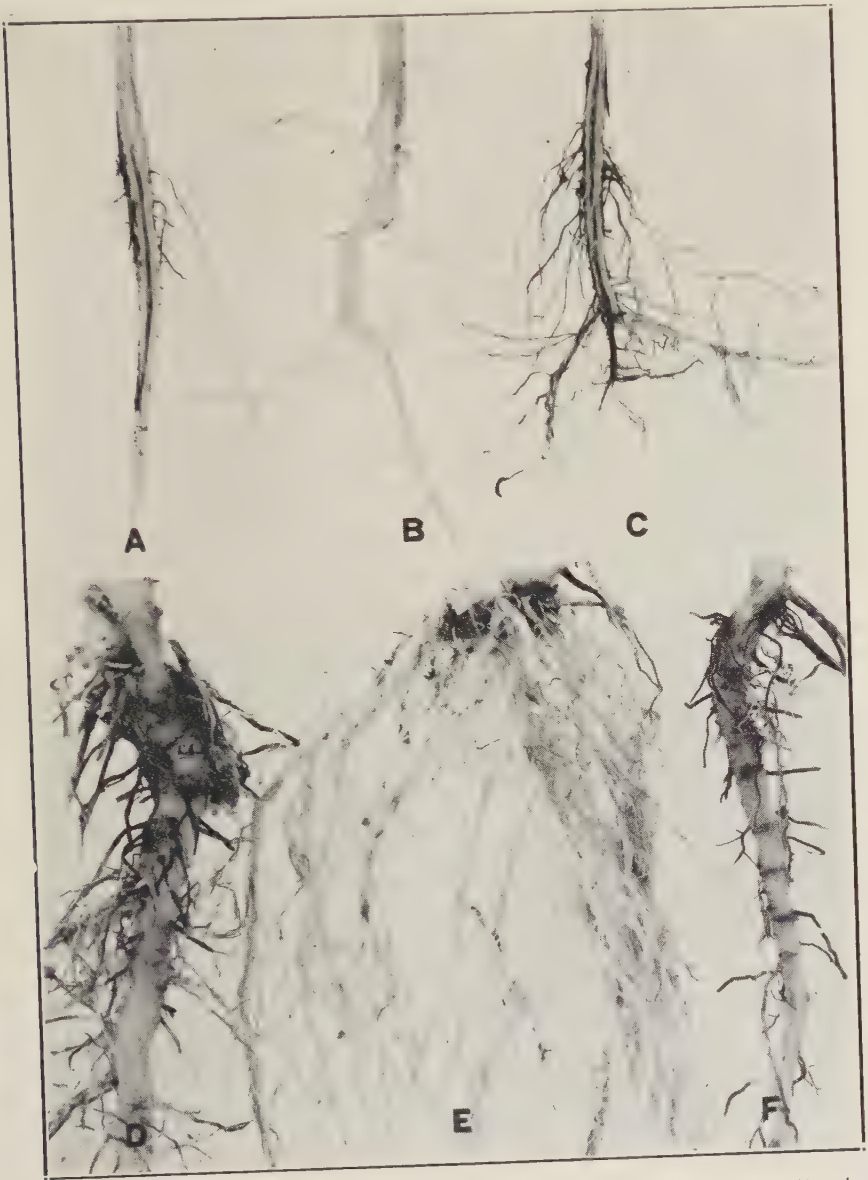

Fig. 3. Cross inoculations with the cotton and tobacco isolates. Pima cotton inoculated with the cotton isolate (A) and tobacco isolate (C). Maryland Broadleaf tobacco inoculated with the cotton isolate (D) and the tobacco isolate (F). Control plants: B, cotton; E, tobacco.

Thirty-seven days after inoculation, a Pima cotton plant was found dead in a sand-culture container inoculated with the cotton isolate, and another in a container inoculated with the tobacco strain. At that time nearly all plants in the inoculated sand cultures showed disease symptoms, and many of the roots were blackened and rotted; only 4 plants finally died. In the soil cultures the Pima cotton plants showed no definite disease symptoms aboveground before March 5. On that date 2 plants inoculated with the cotton isolate were wilted and were found to have rotted roots, but they subsequently recovered. Nearly all of the cotton plants grown in soil and inoculated with either isolate were unhealthy and had diseased roots when examined March 25, but none of them died (Fig. 3, A–C). It appeared that the conditions in the greenhouse were not continuously favorable for optimum development of the fungus in the cotton plants, and the disease symptoms were not so severe on them as it often is under field conditions.

Cross Inoculations

On February 21, both the tobacco and the cotton isolates were reisolated from diseased tobacco seedlings in the greenhouse and cultured on sterilized cotton roots. On February 29, both reisolates were used to inoculate 60-day-old Pima cotton plants grown in soil in the greenhouse in large flats. None of the plants died, but examinations made on March 22 showed similar and about equal damage to the roots from the two fungus isolates. Chlamydospores were abundant in the discolored root tissues inoculated with either.

SUMMARY

A new disease of cotton characterized by a swollen tap root near the collar and an internal purplish-black rot of the vascular tissue was observed at the U. S. Field Station, Sacaton, Arizona, in 1922. The causal organism was isolated in 1938 and identified as the fungus *Thielaviopsis basicola* (Berk. and Br.) Ferraris.

The disease occurs in two widely separated areas in Arizona, but is not believed to be extensive. It persists in the soil year after year, even in the absence of cotton culture. Its spread through the soil is slow and its damage to the cotton crop is not severe, except in occasional seasons. American-Egyptian varieties of cotton proved more susceptible to it than did upland varieties.

The disease is most damaging to the cotton crop in the seedling stage. It sometimes destroys stands of American-Egyptian cotton, and retards the growth of surviving seedlings. Affected seedlings show a characteristic purplish-black dry rot in the central cylinder of the root, where cavities often form. Many seedlings recover when high temperatures prevail, the internal lesions becoming surrounded with healthy tissues. In the fall the occluded lesions may become active and often kill the mature plants.

The disease is readily identified, since microscopic examination of thin sections of the discolored tissues almost invariably reveals the presence of brownish chlamydospores in the conductive tissues.

In cultures on various media the fungus isolated from cotton resembled in appearance and behavior isolates from tobacco obtained from Tennessee and Missouri. Slight differences in color and density of the colonies were apparent on all media, and, on onion agar, the development of white or buff sectors occurred frequently in the cotton isolate but not in the tobacco strain.

Inoculations and cross inoculations on American-Egyptian cotton and Maryland Broadleaf tobacco plants, under greenhouse conditions, were successful with both the cotton and tobacco isolates. Nearly all of the inoculated plants showed symptoms of the disease, either externally or internally, but only a few of them died.

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GALL FORMATION BY PHYTOMONAS TUMEFACIENS EXTRACT AND INDOLE-3-ACETIC ACID IN CULTURES OF TOMATO ROOTS[1]

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(Accepted for publication February 1, 1942)

INTRODUCTION

Since 1936 reports have appeared in which galls incited by *Phytomonas tumefaciens* (Sm. and Town.) Bergey *et al.* have been compared with those stimulated by extracts of this pathogen and by indole-3-acetic acid. The morphological (3, 5, 7, 9), histological (5, 9, 12), and biochemical (11) similarities of these tumors have been observed. Growth hormone is produced in cultures of *P. tumefaciens* and many bacteria and fungi (2, 3, 12, 13, 19, 21), and extracts of the cultured media of the crown-gall organism have given chemical tests for indole-3-acetic acid (12). With a reported exception (15), crown-gall tissue has yielded higher concentrations of phytohormones (auxin a, auxin b, and indole-3-acetic acid) than normal tissue (11, 12, 13). All 3 auxins have been isolated from healthy higher plants (2, 8, 21). In addition, there has been observed a significant increase in the auxin content of legume nodule tissue (10, 20), and of smut-gall tissue of corn.[3] Such results have led to the opinion that the growth of galls, and similar pathological processes, may be related to disturbed hormone balance in the plant (7, 10, 11, 12, 20, 21).

In the present investigation root tips grown *in vitro* were employed as these plant cultures have not previously been used to study gall formation by indole-3-acetic acid and *Phytomonas tumefaciens* extracts. Furthermore, indole-3-acetic acid was employed to ascertain the relationship of growth to tumor development, and to throw light upon a disputed point (2, 4, 6, 14, 16, 21, 23), namely, whether in low concentrations this chemical stimulated or inhibited the growth of roots.

EXPERIMENTAL METHODS AND RESULTS

General Procedures

A strain of excised tomato roots (*Lycopersicum esculentum*) was used upon which considerable physiological data were available (22, 23), and which was very generously supplied by P. R. White. The yeast extract-salt-sugar solution devised by this investigator was employed. In experiments III and IV (Table 1) a synthetic nutrient medium was used in which

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[1] An abridgment of a dissertation in the Department of Bacteriology submitted by the senior writer to the faculty of the Graduate School of Arts and Science of New York University in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Degree conferred June, 1941.

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[3] Moulton, J. E. Doctor's Thesis. University of Chicago, 1941. Quoted by Link and Eggers (11).

TABLE 1.—*The effect of indole-3-acetic acid upon the growth, number of secondary roots formed, and the dry weight of excised tomato roots*

Number of root cultures per concentration	Concentration (mg./l.) of indole-3-acetic acid											
	0		0.00125		0.0125		0.025		0.05		0.5	
	L.[a]	B.[b]	L.	B.	L.	B.	L.	B.	L.	B.	L.	B.
Experiment I												
15	52[c]	4	46	7	19	4	15	6	2	3	1	5
Experiment II												
20	58	7	35	6	17	5	3	1	4	2	0	1

Number of root cultures per concentration	Concentration (molar) of indole-3-acetic acid									
	0		10^{-14}		10^{-12}		10^{-10}		10^{-8}	
	L.	B.	L.	B.	L.	B.	L.	B.	L.	B.
Experiment III[d]										
50	83	14	......	......	71	12	71	11	26	5
Experiment IV[d]										
80	71	10	72	12	75	10	73	11	29	4
Total dry wt. (mg.) Exp. IV	100.3		101.0		100.4		89.6		78.2	

[a] In all tables L. indicates the final length of roots, including length of branches, minus initial length, expressed in millimeters.

[b] In all tables B. indicates the final number of branches formed minus the initial number.

[c] The figure 52 represents the average increase in length in millimeters of 15 roots grown in nutrient solution to which no indole-3-aectic acid had been added (control roots). Similarly, in all tables each number represents an average for each concentration employed.

[d] In synthetic medium.

0.5 mg. thiamin and 1.0 mg. glycine per liter were substituted for the yeast extract in the aforementioned medium.

In subculturing, root tips about 15 mm. long were cut with scissors and removed by transfer needles to 125-ml. Erlenmeyer flasks containing 50 ml. fresh sterile nutrient solution. The root cultures, one to a flask, were kept in dim light at room temperature, and subcultured weekly. Growth was measured by the increase in length measured by millimeter ruler, by the increment in dry weight, and by the macroscopically observed increase in number of secondary roots. In order to obtain greater accuracy in experiments III and IV (Table 1), the rootlets were enumerated under magnification (×100). To determine the dry weight, roots were oven-dried and weighed on an analytic balance. The hydrogen-ion concentration (pH) of culture solutions was measured electrometrically.

To make histological preparations, roots were killed and fixed by Navashin's solution, dehydrated by ethyl alcohol-xylol mixtures, embedded in paraffin, sectioned at 10 μ thickness, and stained by Heidenhain's iron-haematoxylin technic.

Cultures of *Phytomonas tumefaciens* were received from A. J. Riker and E. A. Siegler, to whom we acknowledge our thanks. Galls were formed by these cultures following needle inoculation into stems of young tomato plants, grown at the Brooklyn Botanic Garden through the courtesy of G. M. Reed.

Effect of Culture Extract of P. tumefaciens

The microorganisms were grown 3 days in 6.5 liters of potato-dextrose broth. The culture medium was then passed through a Berkefeld N filter to remove the bacteria. The filtrate was evaporated under reduced pressure to a volume of 100 ml., acidified to pH 3, and extracted 7 times with 0.5 volume of peroxide-free ether. The ether layers were combined and allowed to evaporate at room temperature. The ether residue was added to nutrient fluid in 20 flasks in concentrations of 150 to 1.5×10^{-1} mg. per liter.

As a result, the ether extract reduced the growth of roots, several of the cut ends of which were swollen, and there was excess root hair development.

Effect of Indole-3-acetic Acid

Gross Appearance of Roots. Excised roots are white and translucent, slender and long, regular in shape, and float on the surface of the medium (Figs. 1, A, and 2).

Roots grown in nutrient solution containing indole-3-acetic acid in concentrations from 10.0 to 0.0125 mg. per liter are completely to slightly brown and opaque, stunted in length, thickened and irregular in shape, and submerged in the fluid. Branches appear even at the tip, and like the primary root are stunted in growth. The higher the concentration of the phytohormone, the greater the effect (Fig. 1).

Well-pronounced galls are formed on treated roots (Fig. 3). These are found generally in conjunction with root primordia or branches, but often without the presence of either. The overgrowths may appear on the primary root or branches, at the growing point, medially, or at the cut end.

Microscopic Appearance of Roots. Normal unsectioned roots are regular and of uniform appearance, with a moderate number of root hairs. The vascular stele is readily visible (Fig. 2). As seen from histological preparations (Fig. 4, A), the cells are rectangular, and regularly arranged. The epidermis consists of a single layer of cells. The cortex comprises 3 to 5 layers of parenchymous cells, and a single innermost layer, the endodermis. Underneath the endodermis, a single layer of pericyclic cells bounds the vascular stele. Secondary roots are initiated by meristematic activity of the pericycle.

Treated whole roots are highly irregular in shape (Fig. 3). There is excessive production of root hairs, the tips of which frequently are swollen, like balloons. In microscopic sections (Fig. 4, B–D) of roots treated with indole-3-acetic acid the cells of the epidermis are enlarged; in the higher concentrations of the chemical there is much fragmentation. The cortex is enlarged because of cell hypertrophy, because of increased intercellular space resulting from necrosis and cell disarrangement and of some cellular divisions (hyperplasia). There is intense stimulation of the meristematic activity of the pericycle (Fig. 4, B). Typical thickening in the absence of root initiation is shown in figure 4, D. In the cortex, particularly in the

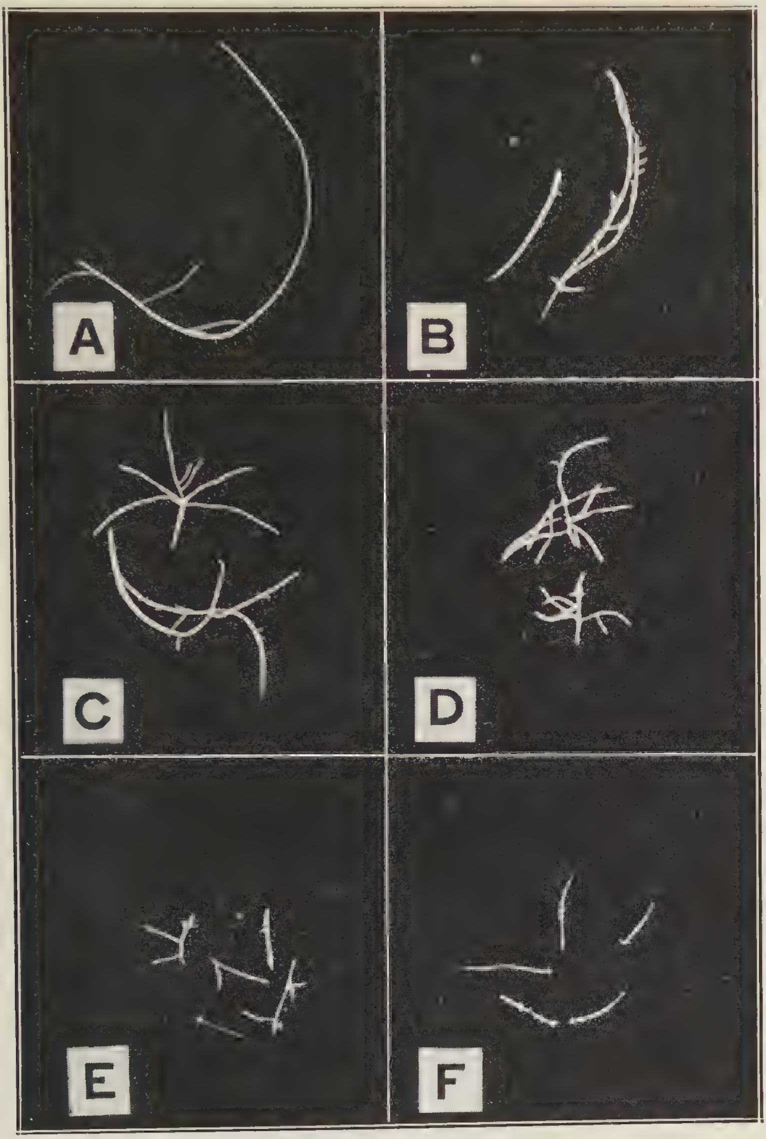

FIG. 1. Effect of the concentration of indole-3-acetic acid on growth of excised tomato roots. ×0.6. A. Normal root. B–F. Roots treated: B. 0.001 mg. indole-3-acetic acid per liter. C. 0.01 mg. per liter. D. 0.025 mg. per liter. E. 0.05 mg. per liter. F. 0.5 mg. per liter.

pericycle, there occur multinucleate cells, most of which are binucleate. Xylem and phloem do not react strikingly to treatment. A comparison of figures 2, A, and 3, E, shows the effect of hormone upon the tips of primary roots. Compare figures 2, B, and 3, C, for the effect upon the cut ends of primary roots.

Growth of Roots. In the previous two sections the pathological effects observed were developed in dilutions of indole-3-acetic acid up to about 7×10^{-8} molar (0.0125 mg. per liter). Up through this dilution, and even to 7×10^{-9} molar, there was unmistakable inhibition of root elongation, reduction in the number of branches formed, and in the total dry weight (Table 1).

To determine if low concentrations of indole-3-acetic acid stimulated or inhibited growth, 2 additional experiments with 600 root cultures were performed. In the first, experiment III (Table 1), the roots grown in normal nutrient solution appeared to have increased in length (16 per cent), and to have formed more secondary roots. In the second test (Experiment IV, Table 1), a concentration of 10^{-12} molar indole-3-acetic acid seemed to accelerate elongation (6 per cent). Dry weight measurements, however, gave almost the same readings for the control, 10^{-14}, and 10^{-12} molar chemical-treated roots, respectively 100.3, 101.0, and 100.4 mg.

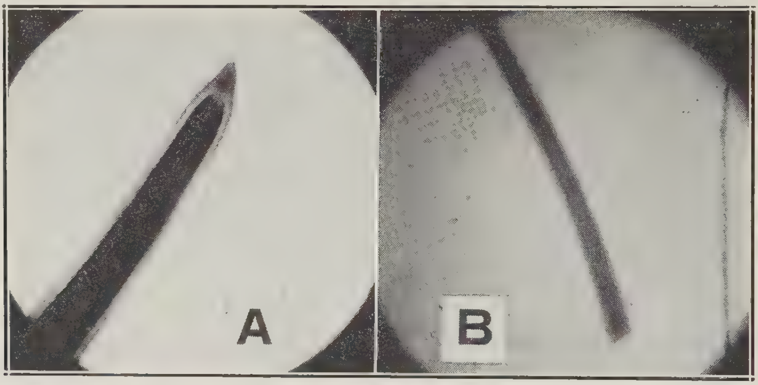

FIG. 2. Normal untreated roots. A. Tip of primary root. ×60. B. Cut end of primary root. ×24.

Toxicity of Indole-3-acetic Acid and Growth of Galled Roots in Normal Solution. An experiment was performed to determine the toxic range of indole-3-acetic acid. After a week's cultivation, the roots were transferred to plain nutrient medium (Table 2). It may be observed that a concentration of 5 mg. per liter is just about lethal, as there is neither elongation nor root initiation, and in addition, only 1 of 10 roots grew after transfers in normal solutions during a period of several weeks.

From the preceding experiment, 6 roots bearing prominent swellings were selected and cultured for 8 weeks in solution containing no indole-3-acetic acid in order to determine whether tumor tissue would grow or would affect the development of new tissue. However, the gall tissue persisted without apparent enlargement and all new growth of galled roots was normal.

Effect of Tryptophane, Indole, Acetic Acid, and pH. As indole-3-acetic acid is an intermediate product in the transformation of tryptophane to indole by microorganisms (2, 21), these substances were tested for their possible carcinogenic effect upon 78 roots. With tryptophane (10^{-4} to 10^{-9} molar) a few swellings resulted, but neither hypertrophy nor initiation of

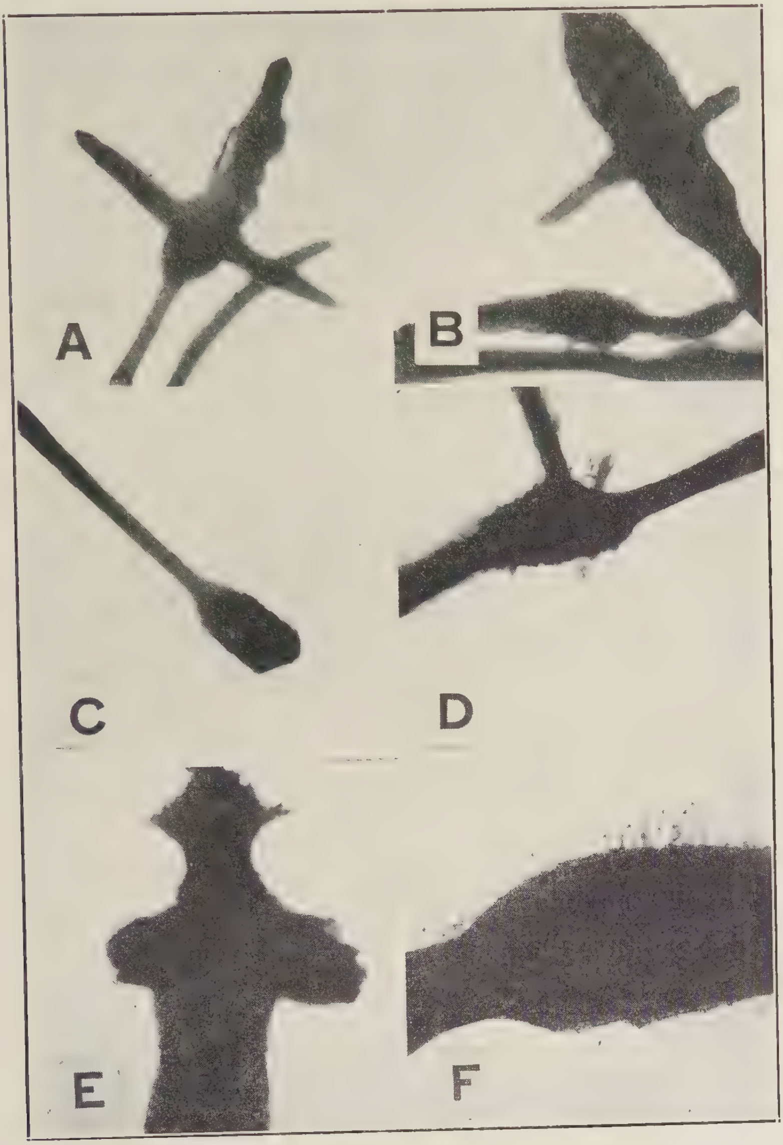

FIG. 3. Galls produced on excised tomato roots by indole-3-acetic acid. A. Root tip and branches in 0.05 mg. indole-3-acetic acid per liter. ×24. B. Root tip and branches in 0.025 mg. per liter. ×24. C. Cut end of primary root in 0.05 mg. per liter. ×24. D. Medial swelling, showing branch and excess root hairs. Root in 0.05 mg. per liter. ×24. E. Root tip treated with 0.5 mg. per liter (retouched). ×60. F. Middle section of root in 0.5 mg. per liter (retouched). ×60.

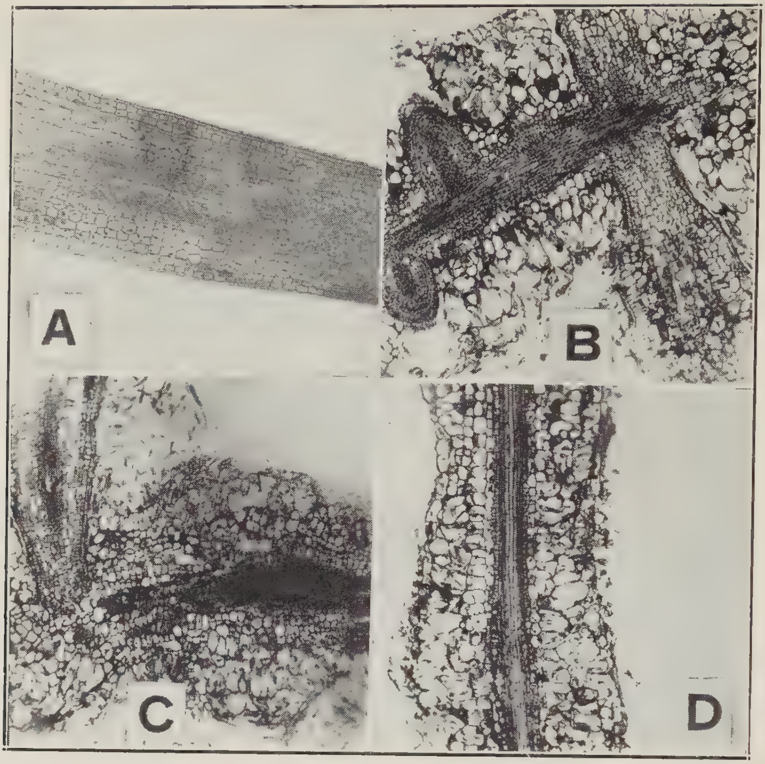

FIG. 4. Histological sections of tomato roots grown *in vitro* in the presence of indole-3-acetic acid. ×100. A. Normal untreated root section. B–D. Treated roots.

root primordia was observed. No effects comparable to those elicited by indole-3-acetic acid were seen when indole (200 to 0.1 mg. per liter) was added to the nutrient fluid.

The addition of acetic acid (10^{-2} to 10^{-8} molar) and of changes made by the addition of potassium hydroxide and hydrochloric acid in the pH of the nutrient solution (pH 0.95 to 11.5) were studied because acetic acid is part of the indole-3-acetic acid molecule, because it has been reported to cause plant tumors (17), and, also, because we have observed in various experi-

TABLE 2.—*The effect of indole-3-acetic acid and transfer to normal nutrient medium upon the growth and the number of secondary roots formed by excised tomato roots*

Number of root cultures per concentration	Concentration (mg./l.) of indole-3-acetic acid							
	5.0		0.5		0.05		0.025	
	L.	B.	L.	B.	L.	B.	L.	B.
10 20—0.025	0	0	0	2	3	2	3	2
Transfer to normal nutrient medium								
Percentage survival	10		60		80		90	

ments that the addition of indole-3-acetic acid increases the acidity of the medium. The results with acetic acid were negative (20 root cultures). In the hydrogen-ion-concentration experiments (121 roots) no swellings were observed, but a few cultures showed increased root hair production, irregularity in shape, and excess development of root histogens; growth was maximal between pH 3.4 and 5.0. In table 3 are given the data from pH 3.0 to 5.7.

TABLE 3.—*The effect of hydrogen-ion concentration upon the growth and the number of secondary roots formed by excised tomato root tips*

Number of roots per H^+ concentration	Average pH of culture medium		Average growth[a]	
	Initial pH after sterilization	pH after culture period	L.	B.
5	3.0	......	0	1
6	3.1	2.8	2	2
2	3.4	......	29	6
5	3.5	......	2	0
4	3.6	......	39	4
6	4.1	5.3	45	4
6	4.2	4.0	54	4
5	4.4	4.7	41	3
6	4.8	......	29	2
6	4.9	4.9	5	1
9	5.0	5.1	4	2
6	5.3	5.5	4	1
5	5.7	6.1	2	1

[a] Cultures were performed at different times of the year, at room temperature, and hence, conditions were different. Such variations affect the growth rate.

DISCUSSION

Although indole-3-acetic acid has been isolated from *Phytomonas tumefaciens* and many other bacteria and fungi (2, 12, 21), we have observed only slight tumor formation following employment of the extracted cultures of *P. tumefaciens*. The weak response noted may be attributed to the use of only 6.5 liters of broth, whereas from 140 liters Thimann (19) obtained only 1 mg. indole-3-acetic acid, and, furthermore, the difficulties of hormone extraction have been observed (11).

Brown and Gardner (3) first clearly demonstrated that structures arising from inoculation of phytohormones into whole plants resembled those produced by the crown-gall organism. The present study reports the production of galls in excised roots cultivated continuously *in vitro*.

The gross and microscopic responses of excised tomato roots to indole-3-acetic acid resembled those induced on intact and decapitated plants by this substance (3, 5, 7, 9, 12, 13), namely, gall and callus formation, inhibition of root elongation, hypertrophy, hyperplasia, root primordia initiation, necrosis, excess root-hair formation, and the presence of multinucleate cells. Similar responses of plants infected with *P. tumefaciens* have been recorded (12, 13, 18).

In this study it was not possible to demonstrate conclusively either stimulation or inhibition of tomato root growth with very dilute concentrations of indole-3-acetic acid (10^{-10} to 10^{-14} molar). In these experiments a synthetic medium was employed to avoid possible introduction of indole-3-acetic acid in yeast extract. With similar root cultures it has been reported (16, 23) that indole-3-acetic acid was neither an essential nor growth-promoting substance; furthermore, tumor production was not mentioned. However, in carrot tissue cultures indole-3-acetic acid (0.000001 per cent) was reported (4) to promote growth, although at higher concentrations cell hypertrophy, root formation, meristematic activity, and callus formation were observed.

White and Braun (24) reported that bacteria-free secondary crown galls could be removed from plants, cultured *in vitro*, and when grafted back into plants crown gall (bacteria-free) resulted. We have observed that upon transfer into normal medium indole-3-acetic acid-root galls have no effect upon the growth of new tissue.

The possible slight activity of tryptophane observed in the present experiments may have resulted from the irritant effect of the toxic concentration employed, or to growth-hormone impurities present in the sample (21), as it is reported to be inactive in the Avena test (2). On the other hand, it is said to be active in the Pisum root formation test (21). Consistent with our observations, indole has not been observed to be an active substance (2, 21). Acetic acid and alteration of pH of medium failed to induce gall development. It has been stated that acetic acid is inactive in the pea test (21), that alteration in the pH of nutrient medium by this acid had no influence upon the growth of roots and root hairs (14), and that pH changes by hydrochloric acid did not affect inhibition of growth as indole-3-acetic acid did (6). Various acids, including acetic acid, have been reported to cause tumors (17), but acid effects, in certain cases, were believed (1) caused by conversion of phytohormone, already present in the plant, from an inactive to an active form.

In the present investigation overgrowths were shown to be incited only by concentrations of indole-3-acetic acid, which actually inhibited growth. The technic of plant tissue (or organ) culture has permitted a quantitative study of the relationship of growth and tumor formation to the concentration of phytohormone, and lends support to the hypothesis of the role of hyperauxony in the formation of galls (7, 10, 11, 12, 20, 21).

SUMMARY AND CONCLUSIONS

By means of ether extraction of culture broth in which *Phytomonas tumefaciens* had been grown indications were obtained that growth substance was produced during the course of its cultivation.

Excised root tips, growing in nutrient solution to which indole-3-acetic acid had been added, responded by the production of galls. Microscopically, the tumors were characterized by hypertrophy of the cortical and epidermal cells, by marked meristematic activity of the pericyclic cells resulting in abundant formation of root primordia, by some multiplication of cortical cells, by the occasional development of multinucleate cells in the pericycle and cortex, by excess root-hair production with frequent swellings of the tips of the hairs, and by stimulation of callus formation at the cut ends of the roots. In the higher concentrations of the growth substance there was much fragmentation of the epidermal and cortical cells, and, in addition, the roots were thickened and of irregular shape.

Galls were formed only in those concentrations of indole-3-acetic acid that caused inhibition of growth.

Up to dilutions of about 7×10^{-9} moles per liter, indole-3-acetic acid inhibited root elongation, reduced the number of branches formed, and decreased the total dry weight. Concentrations of 10^{-10}, 10^{-12}, and 10^{-14} molar did not seem to accelerate the growth of isolated roots growing in continuous cultivation *in vitro*.

Five mg. indole-3-acetic acid per liter was the approximate lethal dose, killing about 90 per cent of the roots.

The chemical galls persisted after transfer to fresh nutrient solution, but all new growth in such media was normal.

The addition of tryptophane, indole, acetic acid, or variations in pH in control cultures failed to induce the formation of tumors.

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RESISTANCE TO MOSAIC VIRUS IN THE CUCUMBER[1]

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For the last 12 years various workers have attempted to develop mosaic-resistant cucumber varieties of high quality. This aim was not satisfactorily achieved. Moreover, the genetical mechanism involved in the manifestation of this disease seemed to be rather complex.

Porter (8) published the first paper on the reaction of cucumber to mosaic. In his breeding work he used the resistant variety Chinese Long, which he procured in China in 1925. According to Doolittle, *et al.* (2), breeding studies for mosaic and wilt resistance were initiated by the United States Horticultural Station at Beltsville, Maryland, in 1929. At this station both Chinese Long and Tokyo Long Green have been used as resistant parents.

Probably one of the greatest obstacles encountered in breeding cucumbers for resistance to mosaic is the apparent difficulty in differentiating various levels of resistance in the second and subsequent generations from crosses between resistant and susceptible parents. Thus, in the course of breeding operation for high quality and resistance, the latter character is progressively decreased.

The present preliminary report is concerned chiefly with the reaction of cucumbers to mosaic, the genetical mechanism involved in the manifestation of virus symptoms, and the criteria for detecting various degrees of resistance.

THE FIRST APPROACH TO THE PROBLEM

In April, 1940, F_2 progenies from the crosses China × Abbott and Cobb (A & C) and Shamrock × A & C were tested in the greenhouse for a study of the virus symptoms. Both China and Shamrock were known to be resistant to mosaic under field conditions. Various methods of inoculation were employed at the 3- to 5-leaf stage, the leaf-rubbing technique proving to be the most efficient. This method is similar to that described by Holmes (3).

All F_2 plants from these crosses showed mottling after inoculation, although there was a pronounced difference in the severity of these symptoms. In general, the F_2 progeny of China × A & C was more resistant than that of Shamrock × A & C. Some of the symptoms in the former cross were delayed for a considerable length of time and appeared on leaves located at different distances from the point of inoculation. Moreover, the delayed symptoms were not so severe and occasionally disappeared after a week or two from the date of their first appearance. No constant genetical ratio could be obtained from these experiments.

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[1] Plant Breeding Paper No. 238. The project, Breeding Cucumbers for Resistance to Mosaic, is a cooperative one between the Departments of Plant Breeding and Plant Pathology at the Cornell University Agricultural Experiment Station, Ithaca, N. Y.

[2] Resident Doctor, Professor of Plant Breeding, and Professor of Plant Pathology, respectively.

[3] Thanks are due to Dr. S. P. Doolittle, of the United States Bureau of Plant Industry Station at Beltsville, Maryland, and to Dr. E. M. Hildebrand, of Cornell University Agricultural Experiment Station, for their helpful suggestions.

In order to determine the causes of such delay in the appearance of these symptoms, the same progenies and other resistant stocks were divided into groups and inoculated at the following leaf stages: cotyledon, first true-leaf, 2-leaf, 3-leaf, 5-leaf, 8-leaf, 11-leaf and 14-leaf. The results of these inoculation studies brought to light three important facts:

1. The delay in the appearance of symptoms is due primarily to genetical rather than to environmental factors.

2. There are 2 distinct types of symptoms of cucumber virus 1.
    (a) Chlorosis—produced mainly on the cotyledons.
    (b) Mottling—produced typically only on the true leaves.

3. All susceptible varieties are chlorotic at the cotyledon stage and all tolerant stocks fail to develop chlorosis on their cotyledons. Therefore, *the criterion for tolerance is the failure to produce chlorosis on the cotyledons following cotyledon inoculation.* As a result of these discoveries some changes in the breeding program were initiated in January, 1941, and some genetical data also were obtained.

EXPERIMENTAL MATERIAL

The following varieties were selected because of their practical and genetical interest:

1. *Chinese Long.* This inbred was obtained from R. H. Porter, of the Iowa Agricultural Experiment Station, and the variety has been described by Porter (8). This particular strain had been selfed previously for a few generations but inbreeding was continued for an additional four generations.

2. *China.* Obtained from Joseph Harris Seed Company, Rochester, New York. This variety is closely related to Chinese Long.

3. *Shamrock.* The variety was originally developed by R. H. Porter. Stocks were obtained from Porter, Vaughan's Seed Store, and A. E. Hutchins of the Minnesota Agricultural Experiment Station. One improved selection was developed at the Cornell University Agricultural Experiment Station from seed of the original Shamrock furnished by Dr. Porter.

4. *Abbott and Cobb (A & C) and Early Russian.* Seed obtained from J. C. Robinson, Waterloo, Nebraska. These are two of several susceptible varieties tested. Both were selfed for two generations before crosses were made.

The first three of the above varieties are known to be resistant to mosaic.

GREENHOUSE WORK

In January, 1941, crosses were made between the resistant parent Chinese Long inbred and the susceptible varieties Early Russian and A & C. The first-generation hybrids were tested and grown during the period March to

May, and F_2 seedlings were tested early in the spring of the same year before transfer to the field.

Method

Cucumber seeds in moist paper toweling were incubated at about 34° C. The seeds germinated after about 24 hours, and sprouting seedlings were planted directly in flats, 2-in. clay pots, or in $2 \times 2 \times 2\frac{1}{2}$-in. paper bands for transferring later to the field.

The plants were subjected to a 15-hour photoperiod (10 hours of daylight and 5 hours of artificial light) up to the set of the first fruit. The average air temperature was about 75° F. Extreme fluctuations of both temperature and light intensity were, however, the rule rather than the exception. During the incubation period, which lasted from 4 to 7 days, the temperature was maintained at approximately 85° F.

The original source of the virus was obtained from a diseased cucumber fruit. Young infected seedlings were used as inoculum. The virus symptoms on the true leaves were identical to those described by Doolittle (1) for cucumber virus 1.

The various stocks, F_1 and F_2 plants were all inoculated by the rubbing technique at the cotyledon stage. When inoculated, the cotyledons were well expanded, but the first true leaf was not yet fully developed. The tested plants were always inoculated together with a check series, comprising at least 100 susceptible plants. Because many inoculations were not very successful the data for the genetical analysis considered significant when the non-chlorotic plants of the check did not constitute much more than 10 per cent. The data from the F_2 were then adjusted.

EXPERIMENTAL RESULTS

Data on the reaction of the different stocks and hybrids to cucumber virus 1 are summarized in table 1. Inoculation was made at the cotyledon stage. Under greenhouse conditions all nonchlorotic stocks and hybrids, in contrast to the chlorotic varieties, were able to make growth beyond the seedling stage. The same varieties, stocks, and hybrids were inoculated and plants were subjected to subsequent infection during their life span in the field. It was possible then to distinguish at least 5 major groups in respect to the resistance of these plants. It is not yet established whether or not inoculation at the cotyledon stage only is sufficient for the demonstration of all these groups. However, in the case of the F_1 hybrids of the cross Chinese Long × A & C it was found that if cotyledons are inoculated and plants are protected from subsequent infection the symptoms may first appear on the third true leaf. It is possible, although no definite proof is available, that the virus invades all the resistant plants, and the presence or absence of symptoms depends on the age of the plant and the genotype involved. A brief description of the 5 major groups follows (Table 1 and Figs. 1 to 4).

1. *Symptomless.* Failure to develop symptoms during the life span. The inbred strain of Chinese Long, obtained from Dr. Porter, is probably

TABLE 1.—*Reaction of different cucumber stocks and hybrids to the virus. All were inoculated at the cotyledon stage and then seedlings in which virus infection was abundant were transferred to the field*

Variety or cross	Cotyledons	First true leaf	Subsequent leaves	Other symptoms and reactions	Field reaction
1. Chinese Long Inbred (Porter)	Nonchlorotic	Green	Green	....	Symptomless
2. China (J. Harris Co.)	do	do	Mild mottling on 8–12 leaves. Eventually plants overcome these symptoms	....	Highly resistant
3. Chinese Long × Early Russian (F_1) (Cornell)	do	do	The third true leaf is mottled. Plants eventually overcome the symptoms	....	Resistant
4. (a) Shamrock R (Cornell)	do	Mottled	Mildly mottled or plants overcome symptoms	Stunting is not manifest	Tolerant to resistant
(b) Shamrock (Porter)	do	Mottled	Mildly mottled or plants overcome symptoms	Slight or no stunting	Tolerant
(c) Shamrock (Minn.)	do	Severely mottled	Mild to severe mottling	Stunted	Low tolerance
5. (a) Early Russian (J. C. Robinson)	Chlorotic	Severely mottled, curled downward	Severely mottled	Severe stunting. Plants remain at seedling size	Highly susceptible. Plants do not reach maturity
(b) Abbott & Cobb (J. C. Robinson)	do	do	do	do	do

homozygous for this character. Whether or not the virus actually invades this inbred was not determined.

2. *Highly Resistant.* Failure to develop symptoms up to about the 8- to 12-leaf stage. The variety China falls into this category. The plants recover from the relatively mild symptoms after a week or two from the date of their first appearance. Occasionally a few plants may show very mild symptoms at the 1- or 5-leaf stage. This may be due to the heterozygosity of the variety.

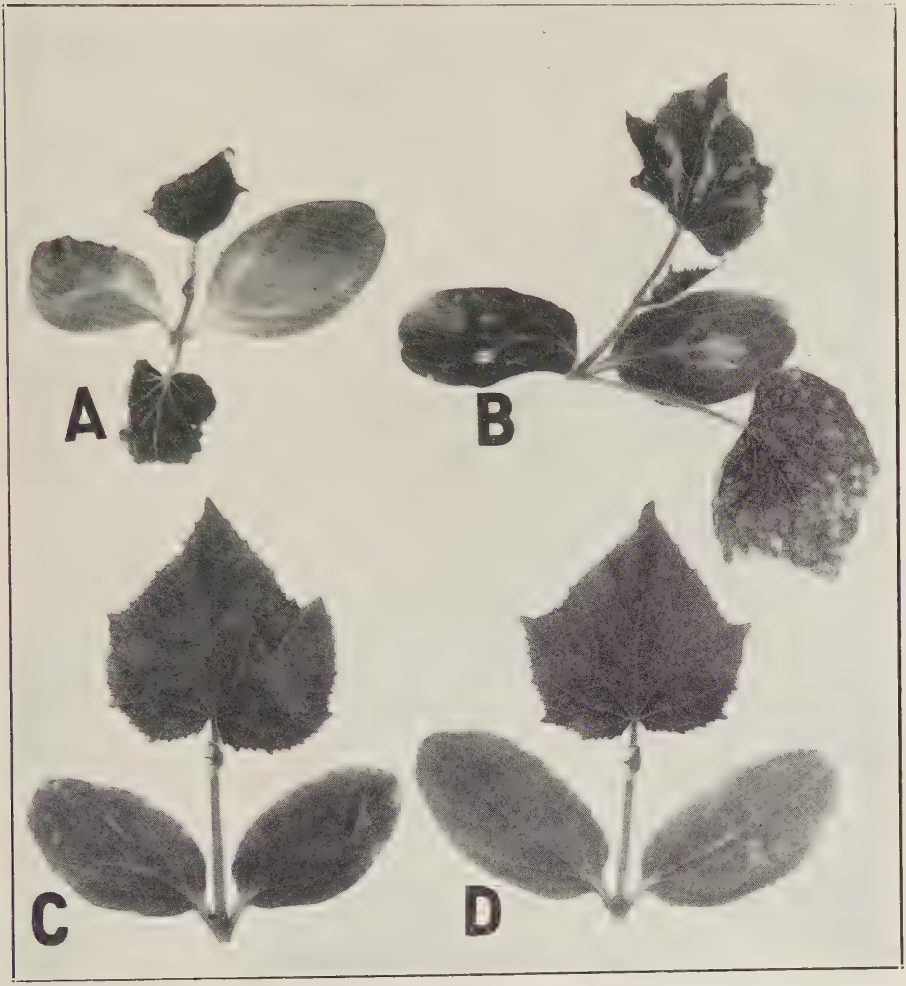

FIG. 1. Reaction of genetically different cucumber seedlings to cucumber virus 1. A. Susceptible. B. Tolerant. C. Resistant (F_1 of symptomless × susceptible). D. Symptomless (right). Under very favorable conditions the F_1 hybrids show mild symptoms on the first or second true leaves.

3. *Resistant.* Failure to develop symptoms up to about the 3-leaf stage. All hybrids of *symptomless × susceptible* may fall into this category. The plants recover from the mild symptoms but new symptoms may appear again

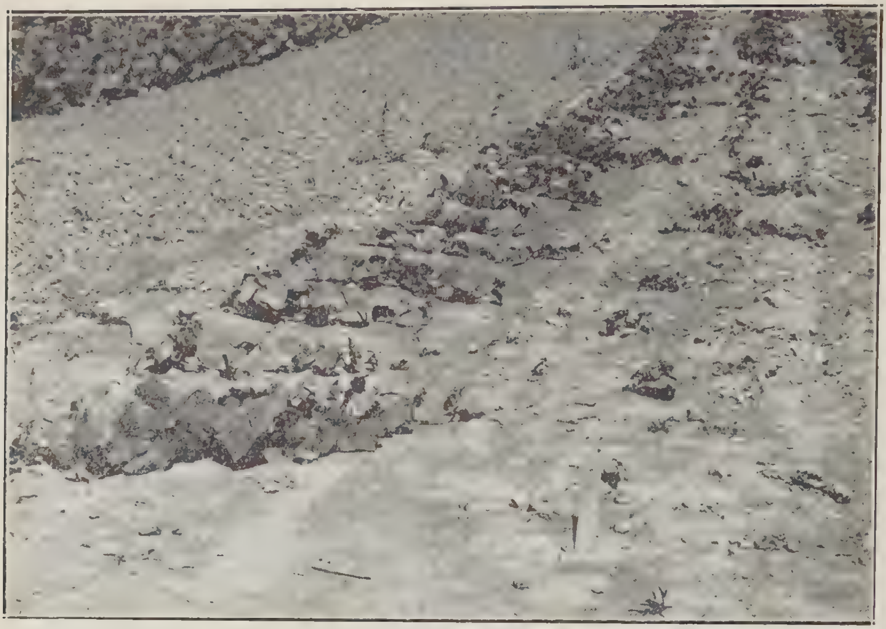

FIG. 2. Reaction of the resistant variety China (left) and the susceptible variety A & C (right) to cucumber virus 1 under field conditions. Both varieties were inoculated at cotyledon stage.

and subsequently disappear. Under very favorable conditions for virus multiplication mild symptoms may appear on the first true leaf of these hybrids.

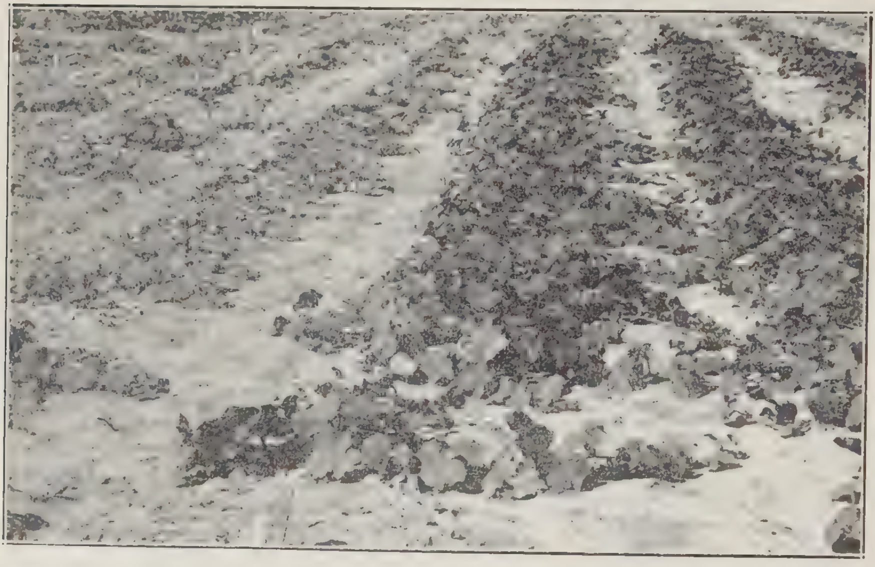

FIG. 3. Resistance to cucumber virus 1 is dominant from a practical standpoint. The two rows with luxuriant plant growth were planted to hybrids symptomless · susceptible. At the left side note the comparative response of tolerant and susceptible stocks. All plants were inoculated at cotyledon stage.

4. *Tolerant.* Failure to develop chlorosis on the cotyledons. Symptoms on first true leaf may be mild or severe, at least for a given period of time. The data in table 1 indicate that all strains of Shamrock have these characteristics. However, they differ in their ability to overcome the symptoms and to reach maturity without great loss in yield. The most tolerant strains of this group do not show any symptoms on the fruit (Fig. 4) and in this respect they possess this important characteristic of the resistant groups.

5. *Susceptible.* Ability to develop chlorosis on the cotyledons. All commercial varieties produce chlorosis following cotyledon inoculation. Chlorosis is followed by severe mottling and stunting of the plants. Under greenhouse conditions the plants may be killed within a short period or remain stunted at the seedling stage for a long time, becoming rather "woody" in appearance and never reaching maturity. Also in the field these stunted plants do not reach maturity but they do not die as do vigorously growing plants which become infected at a later stage. If susceptible plants are infected at maturity the fruits may show mottling and are unmarketable (1).

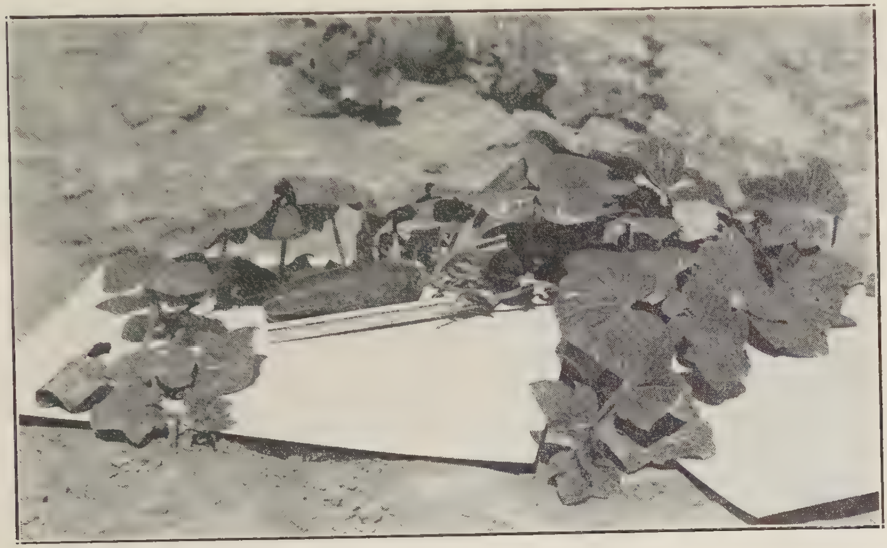

FIG. 4. A tolerant selection (F_5) from a cross Shamrock × A & C. Although some mottling is present on the leaves the plant is not very stunted and fruit is not impaired. This is an excellent slicing type. (See seedling reaction of the same stock in figure 1.)

THE GENETICAL MECHANISM

Very meager data have been published on the genetical control of plant viruses. Holmes (4, 5) described four interesting types of responses to tobacco virus 1 (distorting strain) in *Capsicum frutescens*, which he attributed to a series of allelomorphic genes. Nolla (7) found that in tobacco inheritance of reaction to tobacco mosaic is controlled by duplicate genes and that resistant plants have the double recessive genes. Holmes (6) concluded that in *Nicotiana* the ability to produce necrotic primary lesions is due to a single dominant gene, and the ability to produce systematic chlorosis is due to the recessive allele. In this case the conclusion was based on data obtained from a cross between *Nicotiana digluta* (necrotic type) and *Nicotiana tabacum* (chlorotic type). The F_1 has two sets of *tabacum* chromosomes which synapse and one independent set of *glutinosa* chromosomes (24TT + 12G). From such breeding material one could not expect to obtain a Mendelian ratio or to prove genetically the allelomorphic nature of the characters necrosis and chlorosis.

Porter (8) suggested that resistance to cucumber mosaic in the cucumber is due to one or a few recessive genes. Although not valid, such a conclusion may be made if data be obtained from growing F_2 seedlings only.

In order to gain a satisfactory understanding of the genetical mechanism involved in the manifestation of cucumber virus 1, it is essential to consider 2 distinct developmental phases: 1. *The cotyledon phase,* in which only the reaction of the cotyledons is considered. 2. *The composite true-leaf phase,* in which the response of any given leaf stage is referred to.

It was definitely established that resistance, as indicated by the failure to produce chlorosis, is dominant at the cotyledon stage. First generation hybrids of symptomless × susceptible, resistant × susceptible, and tolerant × susceptible react similarly in this respect.

As these hybrids reach the true-leaf stage they invariably show mottling on their leaves. In these hybrids, however, the adverse effect of the virus varies from no pronounced effect to a relatively marked stunting, depending on the genotypes involved. The hybrids of Symptomless × Susceptible manifest very mild symptoms, which soon disappear and are not affected to any appreciable degree by the invasion of the virus. On the other hand, hybrids of tolerant × susceptible are somewhat stunted at early stages, although eventually they do overcome this condition.

A summary of the data on the reaction of F_2 seedlings at the cotyledon stage is presented in table 2. The observed ratio between nonchlorotic and chlorotic plants is in good agreement with the theoretical 27 : 37 ratio, indicating the action of 3 complementary genes. Only 48 plants of the backcross F_1 (symptomless × susceptible) × *susceptible* were inoculated at the cotyledon stage, together with a check series of 61 seedlings. The check series became 100 per cent chlorotic, while 6 plants of the backcross population of 48 were nonchlorotic. Although this is a perfect 1 : 7 ratio, as would be expected on the basis of 3 complementary genes, the size of the population is rather small.

Thus, at the cotyledon stage, the F_2 population is divided into chlorotic and nonchlorotic groups. As this F_2 population reaches the true-leaf stage the ratio 27 "resistant" to 37 "susceptible" is constantly changing during development. Of 523 F_2 plants only 3 were suspected to be symptomless through their life span. It is apparent that any attempt to secure a Mendelian ratio from growing F_2 plants is futile. Not only the age of the plant but temperature and other environmental factors have a pronounced effect upon inhibition or stimulation of the appearance of symptoms during development. Moreover, some plants seem to overcome the previous symptoms and will, therefore, be classified erroneously as symptomless. It appears that in order to secure reliable data relative to the total genes involved in the manifestation of mosaic symptoms, it is essential to keep weekly records on each F_2 plant at least to the stage in which symptoms first appear.

The fact that only 3 symptomless plants were recovered out of 523 F_2 plants may suggest a 1 : 63 ratio to correspond with the initial 27 : 37 ratio at the cotyledon stage. This conclusion, however, cannot be justified, because it is possible to isolate several homozygous races which vary in their degree of resistance. On the basis of 3 complementary genes there can be only 1 homozygote for resistance; therefore, it is necessary to assume the presence of 1 or several gene modifiers in addition to the 3 basic genes.

TABLE 2.—*Segregating F_2 population resulting from a cross between Chinese Long inbred, resistant to virus, and Early Russian inbred, susceptible to virus. All seedlings including the susceptible check plants were inoculated at the cotyledon stage. Only the characters nonchlorotic vs. chlorotic cotyledons are considered in the genetical classification. Total number of F_2 plants, 812*

Notation of cross	Cotyledon	Observed number of plants	Susceptible check plants	Adjusted numbers (A)	Calculated numbers (27 : 37) (C)	Difference (C – A)	D/P.E.
CE1	Nonchlorotic	176	18	152	165	13	2.0
	Chlorotic	216	164	240	227		
CE2	Nonchlorotic	188	7	171	177	6	0.9
	Chlorotic	232	93	249	243		
Total 812	Nonchlorotic	364	25	320	343	23	2.4
	Chlorotic	448	257	492	469		

These modifiers, it is assumed, are chiefly effective only after the cotyledon stage. The homozygous strains that vary in degree of resistance have either the 3 dominant genes and all the recessive modifiers, or the 3 dominant genes and 1 or more dominant modifiers. The true symptomless plant is assumed to be homozygous to the 3 basic dominant genes and to the maximum dominant modifiers present. The true ratio between symptomless and symptomatic plants is not 1 : 63, but at least 1 : 255 or 1 : 1033.

The F_2 chlorotic group also is not uniform in its reaction and, as a whole, is less susceptible to the virus than is the check; in fact, very rarely is a chlorotic plant found in this group that is so severely affected by the virus as is the check plant. This evidence further substantiates the thesis of multiple factors as applied to this case. The true susceptible plant may be assumed to be homozygous to the 3 basic recessive genes and to the maximum recessive modifiers.

Thus it appears that the genetical mechanism involved in the manifestation of virus symptoms in the cucumber follows a developmental pattern. At the cotyledon stage 3 complementary genes are responsible for the ability or the failure to produce chlorosis. As the F_2 population reaches the true-leaf phase, the initial genetical ratio is constantly changing during development. At this phase several gene modifiers, in addition to the 3 basic genes, also play a role in the manifestation of virus symptoms. As a result the frequency of symptomless plants is exceedingly low.

Criteria for Measuring Resistance

In cases where resistance and susceptibility cannot be regarded as distinct qualitative characters, a general method for detecting degrees of resistance is of paramount practical importance. Nolla (7), working with tobacco, determined degrees of resistance to tobacco virus by the relative number of local lesions produced by the infected plants on *Nicotiana glutinosa, Nicotiana repanda,* and *Nicotiana glutinosa* × *Nicotiana tabacum.* By this method he discovered that the symptomless plants carried the active virus also.

In the case of the cucumber the above procedure is not possible, because cucumber virus 1 will not produce local lesions on *Nicotiana glutinosa* or other known plants. However, we do have a reliable and very simple method for detecting degrees of resistance to mosaic in the cucumber. The first requirement of this method is *cotyledon inoculation.* The criterion for tolerance is the failure to produce chlorosis on the cotyledons. All tested plants that are non-chlorotic are subject to further observation during seedling development. The degree of tolerance is determined by the relative distance from the inoculated cotyledons to the true leaf on which symptoms appear. The greater the distance the greater is the resistance. The above statements should not be considered as a mathematical formula. They do serve, however, as a guide for the breeder. Temperature and other environmental factors have great influence on the appearance of symptoms, and therefore in making decisions one should also be guided by the severity or mildness of the symptoms and by the rapidity with which plants overcome them.

SUGGESTIONS TO PRACTICAL BREEDERS

In order to produce symptomless varieties of cucumbers it is essential to use a resistant parent of the Chinese Long type. On the basis of an F_2 ratio of 1 : 63, not less than 460 plants should be grown in order to obtain, with a reasonable degree of assurance, 1 symptomless plant. However, as has been shown in this paper, more genes are involved in the inheritance of resistance to mosaic and therefore a much larger F_2 population should be grown. For example, a minimum of 7,450 plants must be grown if five genes are involved and 119,270 if seven genes are responsible for the inheritance of resistance to this disease. The task of isolating symptomless plants is not so difficult as it may appear. At the cotyledon stage it is possible to discard about 57 per cent of the F_2 seedlings after inoculation. Within a period of six or seven weeks more than 90 per cent of the total F_2 population can be eliminated. The seedlings can be grown in the greenhouse or in the field. In the latter case they should be planted very thickly, all symptomatic plants being discarded at weekly intervals. Several thousand plants can be handled in this way without much difficulty. Repeated crosses of symptomless selections with the commercial types will result in isolation of desirable symptomless varieties. The two great advantages of this method are:

1. A symptomless variety will prove to be more desirable in the long run from an economical point of view. Tolerant varieties are more apt to furnish the basic material for virus mutation and source of inoculum.

2. The breeder can be sure that his selections are always homozygous for resistance and will breed true for this character. Other breeding methods can be used, the important consideration being that each selfed generation must be subjected to cotyledon inoculation and selected plants preferably should be symptomless up to the 3-leaf stage. By this inoculation method high resistance can be maintained during the breeding program.

SUMMARY

The genetical control of virus symptoms in the cucumber and criteria for measuring resistance are discussed in this preliminary report.

In order to understand the genetical mechanism involved it is essential to distinguish between two developmental phases, (1) the cotyledon stage, and (2) the composite true-leaf stage.

1. Three complementary genes apparently control the ability or the failure to produce chlorosis at the cotyledon stage, the genetical ratio in F_2 being 27 nonchlorotic to 37 chlorotic.

2. The ratio of 27 : 37 is constantly changing at the composite true-leaf stage. At this stage several gene modifiers also take part in the genetical control of virus symptoms. Thus, the frequency of symptomless plants is very low.

3. The presence or absence of chlorosis on the cotyledons determines whether the tested plant is susceptible or tolerant to mosaic.

4. The degree of tolerance can be determined by the severity or mildness of symptoms and by the relative distance from the inoculated cotyledons to the true leaf on which symptoms first appear. The greater the distance the greater is the resistance.

5. All resistant stocks possess the three basic dominant genes. They vary among themselves in the relative number of dominant modifiers.

Some suggestions to plant breeders are included in this paper.

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EXPERIMENTAL AUTOECISM AND OTHER BIOLOGICAL STUDIES OF A GALL–FORMING PERIDERMIUM ON NORTHERN HARD PINES[1]

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INTRODUCTION

In view of the relatively meagre extant knowledge regarding aeciospores from pine, capable of repeating infection on pine, Arthur[3] suggested to Snell the possible autoecism of certain forest-tree rusts. In June, 1932, Snell collected aecia of a bark rust on *Pinus banksiana* Lam. at Schuyler Falls, New York. Later, the writer collected aecia from galls on *P. banksiana* (Fig. 1, D) near Schuyler Falls, and aecia from galls on *Pinus rigida* Mill. at Monument Beach, Massachusetts.

All of the material collected was apparently the heteroecious gall-forming *Peridermium, P. cerebrum* Peck,[4] since the uredial and telial stages of *Cronartium quercuum* (Berk.) Miyabe were found by Snell[5] in both localities on nearby oaks.

No reports of autoecious aeciospores on *Pinus banksiana* capable of infecting *P. banksiana* or other pines, or the successful inoculation of *P. banksiana* with repeating aeciospores from other pines are to be found in the literature. The present study concerns the successful inoculation of *Pinus sylvestris, P. banksiana*, and *P. rigida* with aeciospores from *P. banksiana* and *P. rigida*, [6,7] together with other observations of certain pathological phenomena, physiological disturbances, and resistance to the spread of the fungus in infected trees.

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[1] Part of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of Brown University, June, 1935, together with additional data obtained in experiments at the Massachusetts Agricultural Experiment Station. Massachusetts Agricultural Experiment Station Contribution No. 418.

[2] The writer is indebted to Professor Walter H. Snell of Brown University, under whose direction investigations reported here were undertaken, for advice during the progress of the work and criticism of the manuscript. Grateful acknowledgment for criticism of the manuscript is also made to Dr. Alma M. Waterman, U. S. Division of Forest Pathology; Professor J. S. Boyce, Yale University; and Professor A. Vincent Osmun, Massachusetts State College.

[3] Personal communication to Professor Walter H. Snell of Brown University from Professor Joseph C. Arthur of Purdue University.

[4] Arthur (6) lists *Peridermium cerebrum* Peck (in parentheses) and *Cronartium cerebrum* Hedgc. and Long among the 11 synonyms for *Cronartium quercuum*. The principal interest of this paper is not in the nomenclature of the rust, however, nor in its taxonomic position, and the designation of the rust involved as *Cronartium quercuum* is used by the writer without overlooking the fact that later investigations may necessitate a change of specific epithet.

[5] Professor Walter H. Snell, personal communication.

[6] George G. Hedgcock of the U. S. Department of Agriculture stated in a letter that he had tried numerous inoculations on pines with aeciospores of both *Peridermium cerebrum* and *Peridermium fusiforme* and had never observed the production of galls.

[7] In May, 1932, just previous to the experiments described in this paper, Professor Snell inoculated pines with aeciospores of *Cronartium quercuum* from *Pinus rigida* collected in Massachusetts. His experiments have shown no evidence of repeating aeciospores.

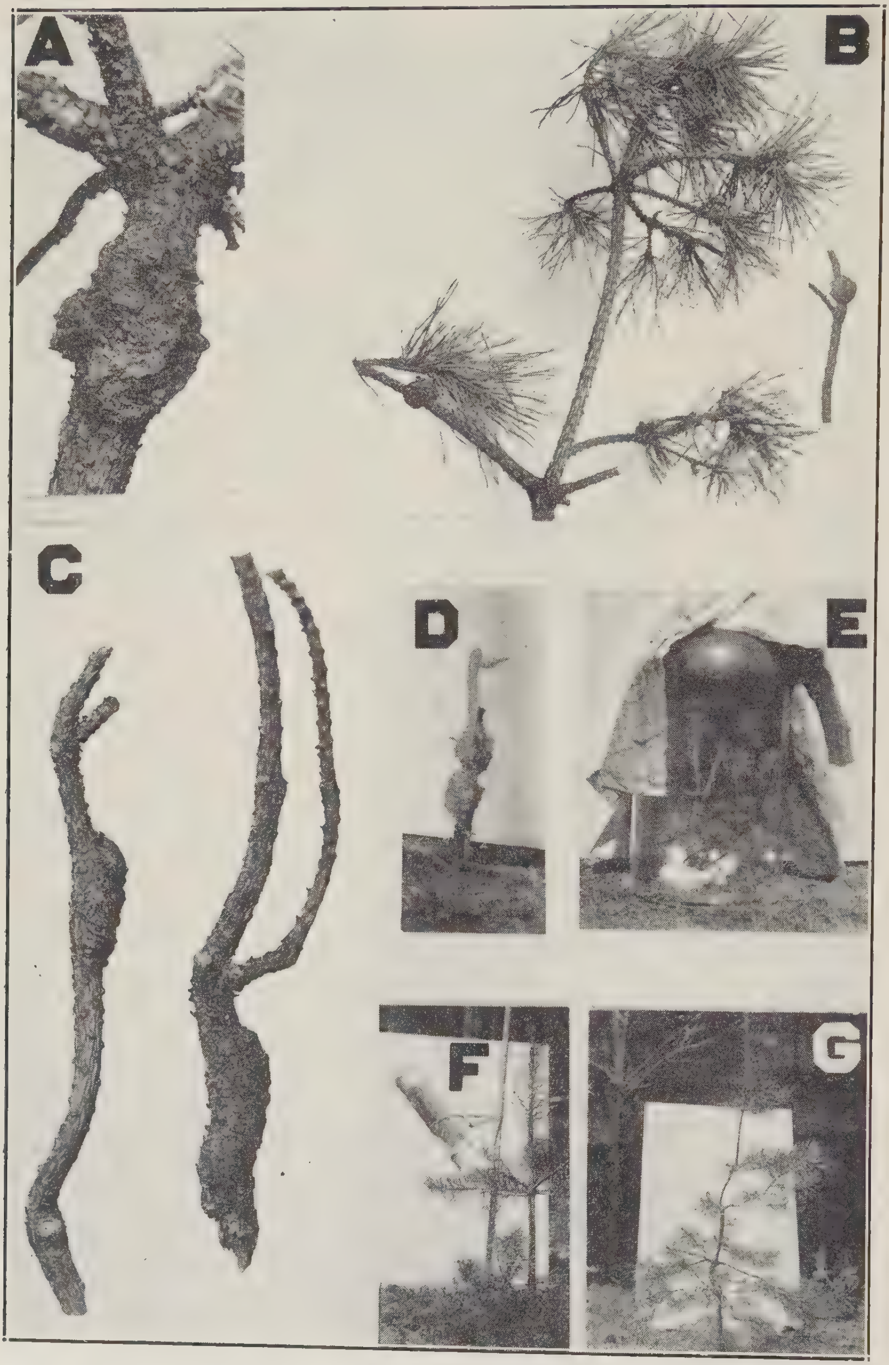

FIG. 1. Galls produced on *Pinus sylvestris* (A, B, C) after inoculation with aeciospores collected from fruiting galls shown in D (galls about 2½ inches in diameter). A and C, approximately natural size; B, somewhat reduced. E. Shaded bell jar used as inoculation chamber. F. Celluloid cylinder used as inoculation chamber. G. 7-year-old check tree of *Pinus sylvestris*.

REVIEW OF LITERATURE

There are few reports in the literature presenting conclusive evidence regarding the autoecism of aeciospores in rusts of forest trees, but these, together with certain of the more numerous reports of investigations on rusts in general, are of considerable interest in connection with the present problem.

Concerning the simplification of the life-cycle of rusts, Arthur (5, p. 184) states "There is a general impression among uredinologists that the length of cycle shows a tendency to shorten in proceeding from the equator toward colder regions of the north." He cites work by Magnus, Ivanov, Morgan-thaler, and Fischer to support this statement and adds "The same proposition is further strengthened in tracing the distribution of the microcyclic rusts." Reports of the heteroecious species of the genus *Cronartium*[8] in which aecia may produce repeating spores include a *Peridermium* on *Pinus attenuata* Lemm., *P. contorta* Dougl., *P. jeffreyi* Balf., *P. ponderosa* Dougl., and *P. radiata* Don. in the western United States (26, 27), and *Peridermium pini* on *Pinus sylvestris* in central Europe (14, 22). Spaulding (33) states: "In 1913 Meinecke [26] made successful inoculations with aeciospores of '*Peridermium harknessii* J. P. Moore' upon *Pinus radiata* under controlled conditions. Later (1920) he changed the name of the fungus to *Peridermium cerebrum* and reported that he had successfully inoculated *Pinus radiata* with aeciospores from *P. radiata* and from *P. attenuata;* and *P. muricata* with aeciospores of *Peridermium cerebrum* from *Pinus radiata.*" In 1929 Meinecke expressed doubt concerning the assignment of the name *Peridermium cerebrum* to the rust referred to in his inoculations and proposed the name *Peridermium cerebroides* (?). He then assigned the name *P. Harknessii* (*Cronartium harknessii* nov. comb.) to the other California pine gall rust, apparently confined to the mountains (28). Arthur (6) refers "*Peridermium harknessii*" (*Cronartium harknessii* Meinecke), *Peridermium filamentosum* Hedgc., and *Peridermium stalactiforme* A. and K. to *Cronartium coleosporioides* and states that the three forms may be treated as distinct species or as varieties under the names *Cronartium coleosporioides harknessii, C. c. filamentosum, C. c. stalactiforme.* Arthur also states: "The form on *Pinus radiata* and *P. attenuata*, sometimes referred to *Cronartium quercuum*, has been shown by Meinecke [26, 27] in many cultures to form repeating aecia on *P. radiata.* Similar cultures were also made by Meinecke [28] with aecia from *Pinus contorta* and *P. sabiniana* sown on *P. contorta, P. radiata, P. Jeffreyi*, and *P. Coulteri.*" The report by Haack (14) that he obtained abundant infection from inoculations of *Pinus sylvestris* with aeciospores of *Peridermium pini* from the same host is discredited by Spaulding (33) because of the conditions under which the experiments were conducted. Under controlled conditions, however, young twigs of *Pinus sylvestris* with and without wounds were successfully inoculated with aeciospores of *Peridermium pini* by Klebahn (22), although

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[8] No heteroecious species of any other genus are known to produce repeating spores in the aecial stage (6).

Liro (24) eleven years earlier had reported that no infections resulted from 169 inoculations of *Pinus sylvestris* with aeciospores of *Peridermium pini. Pinus ponderosa* var. *scopulorum* Englem., *P. contorta, P. sabiniana* Dougl., *P. caribaea* Morelet, *P. mariana* Du Roi, *P. pinea* L., and *P. halepensis* Mill. were successfully inoculated with aeciospores of "*Peridermium Harknessii*" from *P. contorta;* and *P. ponderosa* and *P. virginiana* Mill. were also successfully inoculated with aeciospores of "*Peridermium Harknessii*" from *Pinus ponderosa* by Hedgcock and Hunt (15). Also, a bark rust, "Woodgate Rust," on *Pinus sylvestris,* in which no alternate form is known, produces a gall somewhat like that of *Peridermium cerebrum.*

Spaulding (33) reports that the possibility of autoecism of *Cronartium ribicola* Fisch. (*Peridermium strobi* Kleb.) was considered as early as 1913, "but no evidence of the spread of the fungus directly from pine to pine was found." He cites work by Klebahn, Hennings, and himself in which negative results were consistently obtained.

Galloway (12) reports a microcyclic rust, *Gallowaya pinicola* Arth.,[9] which occurs on the leaves of *Pinus virginiana* throughout the range of the host in the Allegheny Mts. from Maryland to Tennessee. This rust is also reported to have occurred in western Siberia on *P. cembra* L. (6). At one time the correlated macrocyclic species appeared to be *Coleosporium helianthi* (Schw.) Arth. (5), but recently Arthur (6) stated "The correlation of this microcyclic species with a particular macrocyclic form is somewhat uncertain, but it seems to accord well with *Coleosporium inconspicuum* (Long) Hedgc. & Long (*Peridermium inconspicuum* Long), less so with *C. helianthi.*"

In studies of host resistance, Palladin (29) concluded from his work with cereals grown in silicic-acid-deficient solutions that silica in cell walls may have an important part in protecting plants against rust infection. Klebahn (21) considers the difficulty of obtaining successful inoculations with rust fungi in old leaves to be a result of the greater resistance of the cell walls of the epidermis. Arthur (3, 4), Sappin-Trouffy (31), Cobb (9), and Hursh (17) have found that the morphological nature of the host may qualify the development of the mycelia in the host plants. On the other hand extensive investigations by Ward (41), Eriksson and Henning (10), Biffen (7), Vavilov (38, 39), Garber (13), Allen (2), Hursh (17), and Zimmermann (43) have frequently demonstrated that morphological conditions cannot always explain resistance. Hutchinson (18) suggests that resistance to rust infection may be morphological or physiological or a combination of these types.

Various physiological phenomena have been investigated by many observers in connection with infection by rusts. Photosynthesis, transpiration, respiration, and tests of cell contents have been subjects of numerous studies. According to reports, except in the case of "green islands" described by Mains (25) and Rice (30), both gametophytic and sporophytic rust mycelia in host plants cause a decrease in the amount of chlorophyll in the cells and may limit photosynthesis to such an extent as to prevent further development by the parasite. Numerous reports in the literature indicate that transpiration is considerably increased in rust-infected plants, although there are few accurate data available on this point. The various reports concerning the effect of rust infection on respiration agree uniformly in showing a greater intensity of respiration following infection. Except for certain records, which may require special explanation, most observers state that there is a definite relation between the relatively large quantity of starch present during the early growth of the fungus and the diminution of starch as the fungus becomes established. In general, reports agree that all degrees of chlorophyll loss and plastid destruction may result in rust-infested plants. Evidence concerning calcium oxalate in such plants apparently is conflicting, since both increased and decreased amounts are reported (11, 34, 40). Fatty or resinous substances may be present in abundance in rust-infested conifers owing to the increased number of resin ducts, according to Adams (1). Likewise, byproducts resulting from a disturbance of normal conditions in plants may result in maize and sorghum. Rice (30) indicates that various color-producing substances, notably anthocyanin, may occur when these plants are infected.

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[9] Arthur refers *Gallowaya pinicola, Gallowaya pini* Arth., and *Coleosporium pini* Gall. to *Coleosporium pinicola* n. comb.

GERMINATION OF AECIOSPORES FROM COLLECTED MATERIAL

Spores from all galls collected in 1932 and 1934 for the inoculations just described germinated readily for a period of approximately four weeks. After that time germination was erratic. Cooling with ice was found to stimulate germination. Each of the germinated spores was observed to produce from 1 to 3 germ tubes, which became somewhat branched within 12 hours. Under favorable conditions approximately 90 per cent of the spores germinated in 12-hour periods. The optimum temperature for germination in distilled-water drop cultures was 10 to 12° C. Germination did not occur above 20° C. or below 5° C. No spores were observed to germinate later than 10 weeks after collection. Reports on the longevity of spores of *Cronartium ribicola* have been rather completely reviewed by Spaulding (33).

In the sectioned material prepared in the present studies, the cells of the mycelium giving rise to aecia were uninucleate. The spores produced by the aecia were binucleate, and upon germination were observed to form germ tubes, but never basidia (promycelia). The germ tubes of the rust spores under investigation differ from those of the aeciospores of *Cronartium ribicola* in that those of the former are fewer in number and branch less. Otherwise, the present observations and results are similar to those reported for the germination of aeciospores of *C. ribicola* and the germination of spores in Meinecke's experiments on repeating pine rusts. Also, the spores of the woodgate rust from galls on *Pinus sylvestris* have been observed to germinate with a germ tube (True (37)). Therefore, from the results of the germination studies, it would appear that the spores as reported by Meinecke (28), the spores of the woodgate rust from galls on *P. sylvestris,* and the spores concerned in the present study were aeciospores in the proper sense of the term, and not different from the aeciospores of *C. ribicola* and other heteroecious forest-tree rusts, except that the germ tubes of the former will reinfect the aecial host.

EXPERIMENTAL METHODS

In June, 1932, experiments were begun with 12 trees of *Pinus sylvestris* and 6 trees each of *P. banksiana* and *P. rigida,* exclusive of checks; and in June, 1934, experiments were begun with 20 trees of *P. banksiana* and 12 trees each of *P. sylvestris* and *P. rigida,* exclusive of checks. All trees were certified, inspected nursery stock less than 10 years old and were free from physical defects. The pines were inoculated out-of-doors, and no rust or other evidence of infection was apparent prior to the inoculation of the trees. All practicable precautions were taken to limit outside factors.

In the 1932 experiments, the trees were inoculated with aeciospores collected by Snell from *Pinus banksiana* in a forest of natural growth near Schuyler Falls, N. Y. In the 1934 experiments the trees were inoculated with aeciospores collected by the writer in the New York location and also in Massachusetts.

The technique employed in the inoculation of the pines included operations similar to the modification of Hubert's technique employed by York (42) in his inoculations with the woodgate rust fungus and Meinecke's (28) technique in his experiments with repeating pine rusts, and the modifications of Hunt's (16) iceless-refrigerator method described by Snell and Gravatt (32). The details are given here only for the use of celluloid cylinders shaded from direct sunlight.

The trees were inoculated immediately after sun-down by depositing fresh, ripe aeciospores with a sterile camel-hair brush upon wet unwounded and wounded bark of growth of the current season and previous years. Wet cotton wicks were wrapped at the lower limit of the inoculations if the trees were small enough to be covered with bell jars (Fig. 1, E). On larger trees celluloid cylinders (Fig. 1, F) with wet cotton plugs at either end were used to cover the inoculations. All cotton plugs and wicks were kept wet and the inoculated trees were kept in a moist atmosphere, shaded from direct sunlight for four days following inoculation. Some of the checks were given exactly the same treatment as inoculated trees except for the application of the aeciospores, while some checks were left untouched (Fig. 1, G). Most of the material for sectioning was collected from inoculated and uninoculated trees at intervals varying from 24 hours to almost three years during the course of the experiments.

In the preparation of tissues for sectioning, the following killing and fixing solutions (8, 23, 35) were used: osmic acid (2 per cent aqueous solution), picric acid in concentrated aqueous solution, Formalin alcohol (5 parts

Formalin to 100 parts 70 per cent alcohol), Flemming's mixture (an aqueous solution of chromic acid, 0.25 per cent, containing 0.1 per cent of osmic acid and 0.1 per cent acetic acid), Carnoy's solution and Schaffner's chromo-acetic acid solution. The last-mentioned solution was the most successful. Some material was imbedded in paraffin following Zirkle's (44) butyl-alcohol method and later sectioned on the rotary microtome in order to obtain serial sections. Other material was imbedded in nitrocellulose, as outlined by Jeffrey (19, 20), and cut on the sliding microtome. Jeffrey's method was particularly successful in the preparation of gall tissue for sectioning. Free-hand sections were cut directly from freshly collected material and some sections were cut by the ether freezing-microtome from material boiled in water. Transverse, radial and tangential sections 10–20 μ thick were cut in the various ways mentioned.

Three different combination stains were tried on the sections: Delafield's haematoxylin and erythrosin; Flemming's safranin, gentian violet, and orange G; and the orseillin and anilin-blue stain described by Strasburger (36). The stain combinations of Delafield and Flemming proved very satisfactory for the study of uninfected tissue. For the study of mycelium in host tissue the orseillin and anilin-blue combination stain was most helpful.

In connection with the study of normal and infected tissue, microchemical tests were made on freshly cut freehand sections for the identification of cellulose, cutin, lignin, suberin, pectin, starch, fats, gums, tannin, and resin. The tests recommended by Kraemer (23) were followed.

FIELD OBSERVATIONS

All of the inoculated trees of *Pinus sylvestris* and *P. banksiana* and some of *P. rigida* have shown varying responses to infection, including: bark discoloration, gall formation, leaf casting, witches'-brooms, dwarfing, and necrosis. A few of the inoculated *P. rigida* trees developed no symptoms of infection and none of the checks at any time appeared to be infected. Observations showed infection to be limited to the growth of the season of the inoculations. These inoculations resulted in the production of mycelium in all of the species of pine tested, but galls were formed only on *P. sylvestris* inoculated with aeciospores from *P. banksiana*. Seven galls were formed on one 5-year-old tree of *P. sylvestris*. Certain of the galls resulted in the formation of witches' brooms. Numerous investigations of witches' brooms associated with rust infection have been previously reported (5, 6, 28). The galls (Fig. 1, A, B, C; Fig. 2, B) were not uniformly characteristic of any particular rust. Sometimes they were elongated swellings along the stem. At other times they showed indications of forming almost perfect pyriform structures. No aecia or pycnia appeared on the galls, although mycelium was abundant throughout the gall tissues. Arthur (6) indicated that pycnia were usually not found associated with galls in autoecious rusts, although True (37) reported them in woodgate rust. At no time did the young galls appear similar to the typical young galls found in woodgate rust. It should be noted here that reports on the latter rust indicate that its aeciospores will not infect *P. banksiana.*

On all species of pine inoculated with aeciospores from all collections there were observed numerous cases of slight roughening, swelling, or discoloring of the bark, which did not result in the formation of galls. Similar phenomena for other rusts are described by Hutchinson (18) and others. Some cases were observed where the leaders of inoculated pines were very heavily infected and discoloration of the bark was noticeable. In such cases leaf casting and death of the leader, or in the case of the very young trees, of the entire tree, were not uncommon. In still other cases dwarfing resulted. Although the death of cells, organs, or entire plants is comparatively rare in connection with rusts as a group, because of their almost perfect adaption as obligate parasites, necrosis does occur in certain cases. For example, the death of young trees of *Pinus sylvestris* and *Pinus montana* Mill. results from attack by a rust commonly called the "pine twister" so named because the mycelium of the aecial stage causes the shoots to twist about the point of attack. Also, dwarfing or death of trees commonly results when white pines under 20 years of age are infected with *Cronartium ribicola.*

HISTOLOGICAL STUDIES

Histological studies of noninfected tissues of the various pines concerned in the present study showed no important cytological differences between the trees that developed galls, the trees in which rust infection resulted in severe defoliation or death, the trees showing no effect from inoculation, and the checks. Likewise, microchemical tests on fresh sections from the various trees showed no significant differences in the stored food material or in the composition of the cell walls. Certain quantitative variations in fat and tannin were observed, but numerous investigations in addition to the present study indicate that these substances may vary considerably in plants, even in different twigs of the same plant.

In a study of the mycelium in the bark of inoculated pines, the hyphae were always intercellular and followed along the rays past the cambium and into the xylem. In this latter tissue the hyphae were confined chiefly to the ray cells. In tangential sections, the cortex and especially the phloem showed abundant mycelium in strands. Groups of phloem parenchyma cells often were surrounded by strands of hyphae. In both the phloem and xylem rays, hyphae may completely fill the enlarged intercellular spaces, which have resulted from the advancement of the mycelium.

Haustoria (Fig. 2, A) are apparently always unicellular. They were seldom found in young hyphae, but in old infections were observed in all types of host cells, including resin cells and the lumens of the tracheids. The formation of haustoria is thought to indicate a change in the nutritional relationship (5, p. 230) of the parasite and host, but the exact relationship is not known. The haustoria apparently have the power to pierce the cell walls at any point, but no further evidence relative to their function was

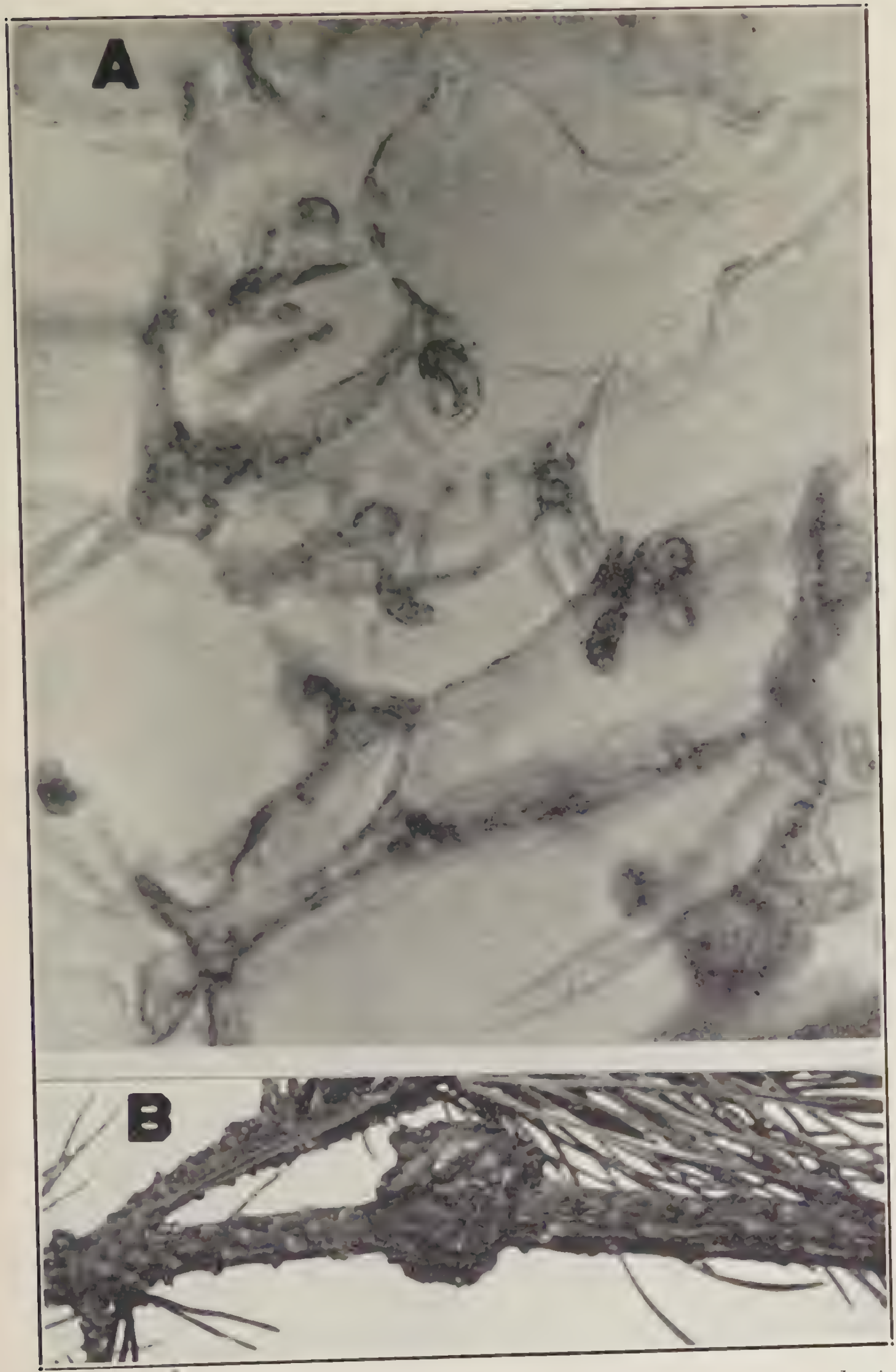

Fig. 2. A. Haustoria in tangential longitudinal section of bark of *Pinus sylvestris*. ×1500. B. Gall from which section shown in A was obtained. ×1.

observed. Young haustoria are usually straight, constricted at the point of passage through the cell wall, and distended inside the cell. In the ray cells the haustoria reach their most complete development.

The host cell and its nucleus showed no evidence that the parasite interfered with the normal growth of the cell. Stimulation toward gall formation was observed, but no relation between haustoria and increased cell division in bark cells was noted. Frequently, haustoria were no more numerous in areas where gall formation was stimulated than in other areas.

Microchemical tests on fresh sections made at the borderline of the advancing mycelium in infected plants showed an abundance of starch, fat, resin, and tannin in tissue recently infected and in adjoining uninfected tissue. In cells apparently infected for a considerable period a marked decrease in tannin was observed.

The examination of sections from inoculated pines in which infection had not resulted in the formation of galls, including all three species of pine used in these experiments, showed two significant host reactions: (1) defoliation, which resulted in no permanent injury to the host, and (2) death of leaders or other new growth. In sections from pines in which defoliation occurred without apparent permanent injury to the trees, rust mycelium was present in dead cortical cells that had been cut off from adjoining tissues by a cork layer. Infection apparently made no further progress in such trees.

In sections from other trees where portions of new growth were ultimately completely killed, mycelium penetrated through the cork tissue into other living tissue where it remained alive for a limited period of time. Many haustoria were produced by the old persistent hyphae. Other cork layers were formed, however, and cut off the infected tissue. Sometimes this process continued for a while, but as the mycelium advanced, the limits of the newly infected area were more narrowed than those of the previously infected area, and finally a cork layer completely surrounded and checked the growth of the mycelium. In no case did sections of trees with dead leaders or sections of temporarily defoliated trees show that the rust had succeeded in attaining the mutualistic relationship in the host necessary for the true establishment of rust mycelium in plant tissue.

In general, the details of the histology of the sections from trees in which rust mycelium failed to become permanently established were similar to those known to occur in tissues of trees recovering from wounds. From the point where the advancement of the rust mycelium appeared to be checked in the host tissue, the histological details in the present study were somewhat similar to those described by Hutchinson for his inoculations of *Pinus sylvestris* with repeating aeciospores of woodgate rust. He found that considerable pathological tissue is formed during the time the rust mycelium is present in the host, but concluded that transient infection indicates resistance of the host. In the trees which he called resistant, however, he reported no such pronounced reactions following infection as occurred in certain cases in the present experiments. Perhaps in the latter, where infection apparently had been arrested, the myclium was not permanently checked and would advance further at some later time. It would then appear that extremely heavy infection was responsible for the reactions of the host resulting in defoliation or death of new growth and also for the delay in the establishment of a balanced relationship between the parasite and the host.

PHYSIOLOGICAL STUDIES

Limited observations were made on the inoculated pines concerning physiological phenomena associated with rust infection. Trees in which the leaders were killed and temporarily defoliated trees, obviously lost considerable chlorophyll-bearing surface and suffered from the limitation of photosynthesis, respiration, and transpiration. Certain chemical differences in cell contents may have had a bearing on physiological reactions. Microchemical tests mentioned under histological studies indicated that there were some chemical differences between infected and uninfected plants. Whether a rapid reaction of the fungus following infection in leaders or other new growth was responsible for the defoliation or death of trees was difficult, if not impossible, to determine. Some unbalanced relationship, however, must have existed after infection in order for temporary defoliation or death to have resulted. In trees in which rust mycelium had spread considerably, physiological disturbances apparently were not serious enough to cause such conspicuous host reactions, but witches' brooms have been formed in some cases. No satisfactory explanation can be given here for the physiological reactions associated with infection.

DISCUSSION

There are on record only three or four species of the pinicolous, caulicolous, gall-forming genus *Cronartium,* the aeciospores of which will reinfect pines and which therefore are microcyclic, obligately or facultatively as the case may be. One of these is the European *Peridermium pini* on *Pinus sylvestris,* for which no alternate host is known. The second is the *C. harknessii-C. coleosporioides* complex on several hard pines (6, 26, 27, 28). Then there is the case of the species on *Pinus radiata* and *P. attenuata* in the western United States (26, 27), doubtfully referred to *Cronartium quercuum* (6, pp. 24, 26, 30). And finally there is the woodgate rust discovered by York on *Pinus sylvestris* in New York State, for which again no alternate host is known, and which was referred by Arthur to *Cronartium quercuum* (6, p. 26) after this investigation was started.

The present studies were undertaken primarily to determine whether the aeciospores collected from pines were capable of reinfecting the pine hosts. Aeciospores from galls on *Pinus banksiana* and *P. rigida* growing within a few yards of oaks bearing uredia and telia were inoculated onto *Pinus sylvestris, P. banksiana* and *P. rigida.* Studies of the spores used in the inoculations leave no doubt that they are true binucleate aeciospores which germinate by means of germ tubes.

If the aeciospores used in these investigations were produced by *Cronartium quercuum*, and this species on the hard pines in the eastern United States has not previously been known to produce repeating aeciospores, the following questions arise:

1. Is the Peridermium stage of *C. quercuum* facultatively autoecious in general? That is, are the aeciospores capable of reinfecting pines instead of, or possibly as well as, oaks, the usual uredial hosts of this rust? 2. Have we here a strain of *C. quercuum* on a certain pine host, which has become confirmedly microcyclic?—that is, one that can infect pines only and oaks not at all? 3. Or have we a purely artificial situation created by the conditions of inoculation experiments? It is regrettable that these questions cannot at present be answered. It can be stated only that it has been found that aeciospores of this rust from two pine hosts in two localities, in both of which infected oak leaves were found, can reinfect the usual aecial host under experimental conditions.

As to the bearing of these results upon the problem of the identity of the woodgate rust, likewise no conclusions can at present be drawn, because the infections and galls produced did not mature sufficiently to enable one to compare them with typical woodgate rust galls. It can be said only that there still remains a possibility that woodgate rust may be microcyclic strain of *C. quercuum,* as Arthur concludes on morphological grounds.

While it must be admitted that few data are available with respect to the prevalence of microcyclic forest-tree rusts, and especially as to facultative microcyclicism in rusts that are normally macrocyclic, and while the data presented in this paper are meagre, on the other hand it is interesting to speculate upon the bearing of these facts upon the evolution of rusts in general. In view of the "general tendency towards shortening of the life cycle . . . in cooler climates and higher latitudes" (5, p. 185), it would be interesting to make observations and perform experiments upon various rusts in their more northerly habitats. *Cronartium quercuum,* for example, should be investigated in Canada. Furthermore, this species should be of special interest because of the possibility of its plasticity with regard to the evolution of microcyclic races or species. Meinecke's successful inoculations with aeciospores of a species on west coast and mountain pines, doubtfully referred to *C. quercuum,* and the successful inoculations reported herein with aeciospores of what may be this species, are cases in point.

Repetition of experiments presented here and further investigations along these lines are desirable from a variety of points of view. Such studies are expensive, time consuming, and difficult to evaluate, but these facts should not be permitted to prevent much needed further critical investigation of the heteroecious rusts of the genus *Cronartium.* At the present stage of our knowledge of the subject, one is entitled to wonder what may be the situation in the other heteroecious forest-tree rusts not investigated, even though failure has marked all attempts to show the repeating character of the aeciospores of *C. ribicola.* Furthermore, it should be kept in mind that there is a very important practical aspect of the subject. Facultative autoecism of aeciospores in an economically important rust would present a problem of grave importance in the practice of forest pathology, since control of the parasite would be materially handicapped by its ability to spread after the elimination of the alternate host.

SUMMARY

Inoculations of *Pinus sylvestris*, *P. banksiana*, and *P. rigida* were made with aeciospores of a gall-forming Peridermium, presumably referable to *Cronartium quercuum* (see footnote 4), from *P. banksiana* and *P. rigida*. The aeciospores used in the inoculation experiments were binucleate. The mycelia giving rise to the aecia that bore the spores were uninucleate. Germination of aeciospores always showed them to form germ tubes. No basidia or secondary spores were observed.

The inoculations resulted in the infection of all hosts. Only in the case of *Pinus sylvestris* inoculated with aeciospores from *P. banksiana*, however, were galls formed. No aecia or pycnia appeared on the galls. On all species of pine inoculated with aeciospores from all collections, numerous cases of slight roughening, swelling, or discoloring of the bark, which did not result in the formation of galls, were observed. Histological studies of discolored bark showed the presence of typical rust mycelia and haustoria.

Microchemical tests made of the trees showing infection have indicated certain differences between the various plants after infection. No significant differences were apparent prior to infection. In some infected plants bark discoloration, abundant mycelia, haustoria, galls, and witches' brooms were observed. In other infected plants defoliation and death of leaders or complete necrosis have resulted.

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THE SPREAD OF VIRUS DISEASES OF THE YELLOWS TYPE UNDER FIELD CONDITIONS

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INTRODUCTION

The various factors related to wind and weather, which vary in an indeterminable manner and which influence insect behavior, probably are considered as insurmountable barriers to a rational consideration of the spread of insect-transmitted diseases and the dissemination of the viriferous[1] agents. However that may be, an understanding of the pattern produced under field conditions would be of obvious value to plant pathologists, and it seems likely that several applications may be made of an adequate description of the disease spread and virus dissemination. In this paper the writers are attempting such a rational consideration of disease spread in a special case of the insect transmission of viruses of the yellows type. Although an evaluation of constants cannot be undertaken, the general equation developed seems to describe adequately the distribution pattern, inasmuch as data we have on hand satisfy the demands of the equation. Unfortunately, observations reported have been restricted to the dissemination of yellow dwarf of potatoes into potato fields,[2] and of the dissemination of aster yellows into endive and lettuce beds,[3] but the results seem interesting enough to publish.

THEORETICAL

In a consideration of the spread of a virus disease by leaf hoppers, there are two extreme cases that may lend themselves to some sort of analysis; the one where the spread from plant to plant within the field is important, and the second where the spread of the disease from plant to plant within the field in question is of no importance and where the bulk of the virus effective in the spread has come from sources outside the field. This situation would be realized if the virus were picked up by the insect with difficulty in the field in question (as, for example, yellow dwarf in a potato field) or if the great majority of invading insects were initially viriferous. By spread from plant to plant within the field we have in mind that increased spread of the disease attributable to the increased percentage of viriferous insects resulting directly from insect feeding on plants that were infected as a consequence of the invasion. A limitation in these deductions is that *only* the case where the spread of the disease from plant to plant within the field is of no importance shall be considered. One may feel justified in considering that the bulk of the effective inocula was brought into the field in the two cases concerned because, on the one hand, observations show that it is infrequent that leaf hoppers become viriferous from feeding on a yellow dwarf potato plant, and on the other hand, there is good reason to believe that the percentage of viriferous aster-yellows leaf hoppers that invaded our various fields was very high.

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[1] So far as we have been able to learn, the term *viriferous* was used initially by C. F. Stahl and Eubanks Carsner (Jour. Econ. Entomology **16**: 476–479. 1923) to connote a virus-bearing agent. They later adopted the term *viruliferous* for the same purpose (Jour. Agr. Res. 28: 297–319. 1924) because it had been used in the medical literature and they wished to avoid the duplication of terms. We like the term *viriferous* and are reintroducing it at the risk of being criticized for nonconformity.

[2] Hansing, E. D. A study of the control of the yellow dwarf disease of potatoes. Ph.D. thesis, Cornell University. Unpublished.

[3] Linn, M. B. The yellows disease of lettuce and endive. New York (Cornell) Agr. Exp. Stat. Bull. 742, 1940.

In any analysis of a mathematical nature some assumptions are necessary in setting up equations. Should the hypothesized phenomena agree well with observation, one may conclude that the assumptions made were reasonable, and that he has given expression to a natural law, which, however, should be accepted only after extensive study. Two basic assumptions are made in the consideration of virus dissemination under field conditions, one of which is that the movements of the leaf hoppers involved is random. The second assumption, which may not stand too close scrutiny, is that the number of plants in a given area that become infected is proportional to the number of insects in that area, to the average number of different plants an insect will feed on per unit of time, and to the time. That is, the number of plants that become infected is proportional to the number that have been fed upon. The difficulty with this assumption is that there is in any case only a limited number of plants per unit area, and that, as time goes on, the number of hitherto unmolested plants becomes less and less, and the probability of an infection per unit time consequently becomes less and less. This factor is disregarded in the following deductions because its consideration gives rise to mathematical difficulties that are indeed formidable, but chiefly because it does not seem to have too great a bearing on the problem, as shall be seen later.

In order to facilitate the mathematical treatment one may direct his attention to an isolated unit area of the field, having its edges parallel to the edges of the field, and consider the events that occur in that area, and then sum up the events for the entire field and for the total duration of the experiment. In the invasion of a field where the movements of the individual insects is random, there will be more insects entering the unit area on the side near the source of insects per unit of time than are leaving it from the opposite side, and there will be, consequently, a net gain in insect population per unit time. The component of the movements of the insects parallel to the edge of the field near the reservoir cancel out, so that one need only consider the flow of insects in the direction perpendicular to the reservoir edge of the field. Mathematically expressed,

$$-\frac{\partial c}{\partial x}K$$

insects enter the unit area per unit time where c is the number of insects per unit area, x is the coordinate in the direction of insect flow, and K is average distance an insect will move in the x direction in our unit time interval, and $-\frac{\partial c}{\partial x}$ is the density gradient. The number of insects leaving the unit area per unit time then is

$$-K\left[\frac{\partial c}{\partial x}-\left(-\frac{\partial}{\partial x}\left(\frac{\partial c}{\partial x}\right)\right)\right]$$

and the difference between these two expressions represents the net gain in insect population per unit time. The time rate of change of insect population density in the unit area may be expressed as

$$\frac{\partial c}{\partial t}=K\frac{\partial^2 c}{\partial x^2} \tag{1}$$

The concept involved in the development of equation (1) is that the migration of insects in mass may be considered as comparable to the flow of a compressible fluid. However, a more rigorous derivation may be obtained from considerations involving some of the principles of the Boltzmann statistics in the case of a 2-dimensional gas, where the average length of insect hop corresponds to the mean free path of the gaseous molecules. Equation (1) is, in reality, the general equation for the diffusion process in one direction, and there is implied a very close analogy between the behavior of the individual insect and the thermal motion of an individual fluid molecule or colloid particle, and K corresponds to the diffusion constant. There may be the objection raised that the assumption involved in the derivation of equation (1) is not valid because of the effects of wind and various other factors on the migration of insects. The answer is that the effects of these various factors appears to be one of superposition, and the general pattern produced over a reasonable period of time is not affected. That is, the general pattern produced will be the same whether the insect migration be rapid or slow, and the equation will hold true for any element of time, providing the movements of the insects be random. The effect of the factors related to weather that affect the insect migration will have an effect on the value of K in equation (1), and the value of K is taken over a long period of time.

Equation (1) has to do with the behavior of the insects, whereas the primary interest in this paper is in the spread of the disease. Since the disease is spread by the insect, there is a very close relationship between the two. The connection between the variation of the insect population and the variation of amount of disease occurring in the unit area may be shown in the following manner. If, for the moment, one may regard the unit area as containing a constant insect population, one may write, in keeping with the second basic assumption,

$$I=cntR \tag{2}$$

where I = number of plants that have become infected in time t
c = number of insects per unit area
n = average number of different plants an insect will feed on per unit of time
t = time
R = proportionality constant.

Now

$$\frac{\delta I}{\delta t} = cnR + \frac{\delta c}{\delta t} tnR \qquad (3)$$

and

$$\frac{\delta^2 I}{\delta x^2} = \frac{\delta^2 c}{\delta x^2} ntR \qquad (4)$$

and one may substitute equations (3) and (4) in equation (1) to obtain

$$\frac{\delta I}{\delta t} - \frac{I}{t} = K\frac{\delta^2 I}{\delta x^2}. \qquad (5)$$

Equation (5) is the differential equation for the spread of a disease under field conditions, subject to the limitations imposed.

The form of the solution of equation (5) that is desired will depend on the experimental set up; in the field of physics the experimental conditions are spoken of as the boundary conditions. In the present considerations we shall take as the boundary conditions: (1) the insect population in the freshly plowed field at the start of the experiment is negligible, (2) the area that serves as a reservoir of insects has a very large supply of insects and that the invasion of the field in question does not appreciably lessen the population density in the reservoir, (3) attention will be confined to regions not too near the edges of the field. Under these boundary conditions a solution of equation (5) that appears satisfactory is

$$I = Kte^{st-\sqrt{s}\,x} \qquad (6)$$

where s and $\sqrt{s}$ are constants of integration and where e is the Napierian constant.

One may, if he desire, divide both sides of equation (6) by the number of plants in the unit area without affecting the validity of the equation, and thus handle the data in terms of fractions of, or percentages of, plants infected.

Equation (6) may be written in the logarithmic form

$$\log I = \log K + \log t + st - \sqrt{s}\,x \qquad (7)$$

and it is seen that two demands made by equation (7) are that, at any given time log I be proportional to the distance into the field, and that, at any point in the field, log I be proportional to log t + t. In the case where the variation of percentage infection is observed for a definite time, $-\sqrt{s}$ is the slope of the curve, and, at the intercept where x = 0, log I = log K + log t + st. In the case where we fix our attention to a given area in the field and observe the variation of percentage infection with time, and plot log I – log t against t, the slope of the curve is s. The value of K may be determined by observing the behavior of the insects in question under field conditions.

EXPERIMENTAL

The several experiments that have been conducted under field conditions permitting a test of these two demands by equation (7) may be described briefly. The first field plot in studies with yellow dwarf of potatoes in up-state New York is shown diagrammatically in figure 1, with the rows planted along contour lines. Between the well-worn lane, at the top of the figure, and the potato plot there was a 10-foot uncultivated strip in which there occurred weeds, clover, and some clover leaf hoppers. Data were obtained by permitting the plants to mature and then tuber indexing representative samples in the greenhouse. The old meadow was densely populated with viriferous clover leaf hoppers. The distance into the field was measured at right angles to the contour lines, and the row width was taken as the unit of length (about 18″). The time was necessarily the full season. In this case, as well as the various others, yellow-dwarf-free seed stock was used.

There was nothing unusual about the other potato plots except that they were adjacent to fields in which virus-bearing clover leaf hoppers[4] occurred.

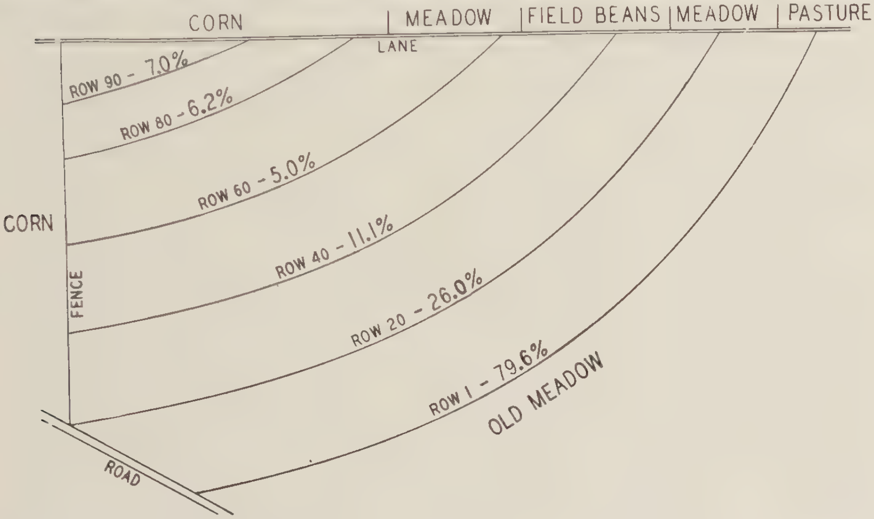

FIG. 1. The field plot in the first experiment on the dissemination of the yellow dwarf virus into potato fields.

The work with aster yellows was carried out on Staten Island, N. Y. A typical field plot is the endive plot shown in figure 2. The weeds to the left served as a reservoir of viriferous aster-yellows leaf hoppers; note that the fraction of plants diseased decreases as the distance into the field increases.

In figure 3 we have plotted log I, where I is the percentage of plants infected with yellow dwarf, against the distance into the various potato fields involved in the experiments. The top curve in figure 1 was the one obtained from the data taken in our first field plot, namely, the field diagrammatically represented in figure 1. The influence of the insects migrating into the field from the lane may be readily seen; there was a decrease in infection from row 90 to row number 60. It is evident from figure 3 that, with the exception of one field, the invasion by the insects was from both edges of each field. Note that in each instance one of the demands of equation (7) is met; log I is proportional to the distance into the field.

[4] For details see the article by Hansing, footnote 2.

The situation is the same in the case where aster yellows spread into endive beds from the adjacent weed patches. Log I, where I is the percentage of plants infected, is plotted, in figure 4, against the distance into the field for four separate experiments. The demand of equation (7) that log I be proportional to the distance into the field is met by these data.

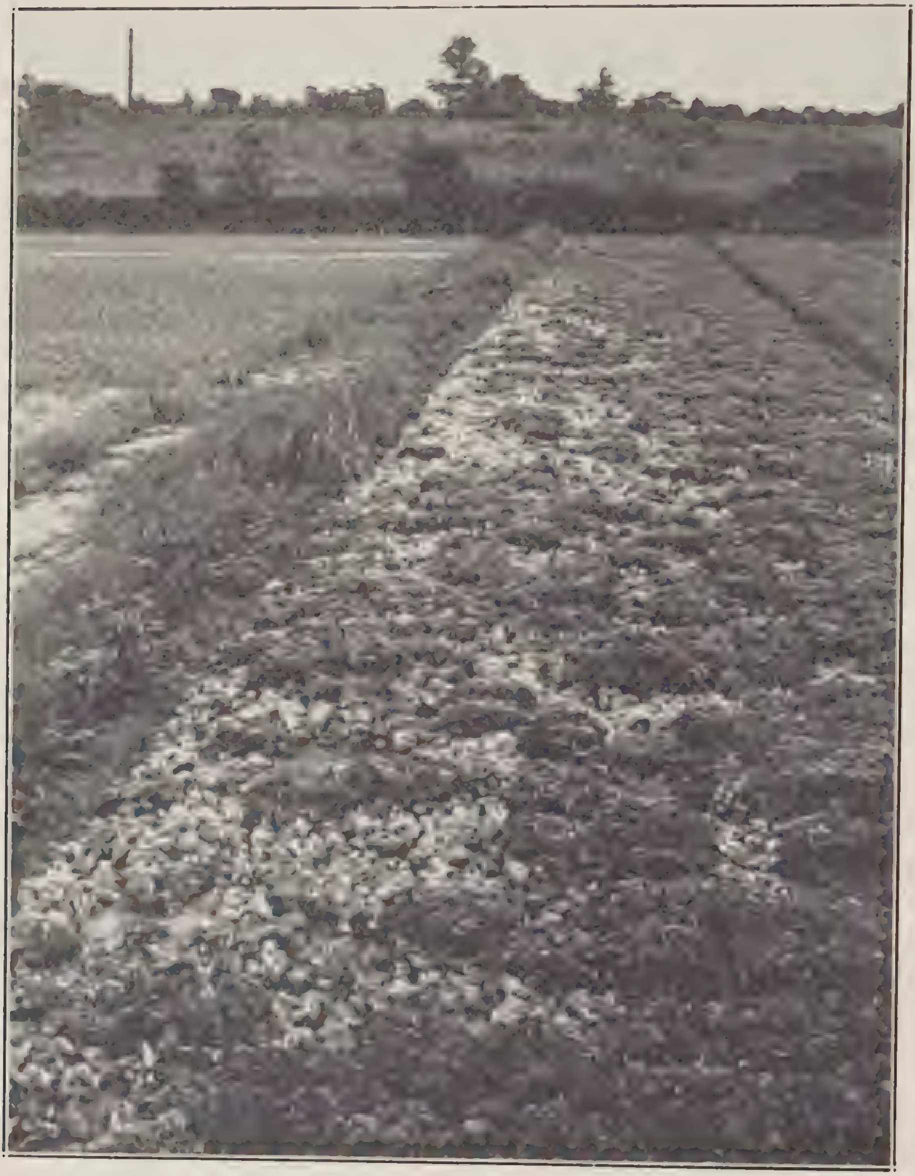

FIG. 2. Appearance of the typical endive bed in the case where the aster-yellows virus has spread from the adjacent weed patch

In any experiment where the insect population is held constant, I = cntR,

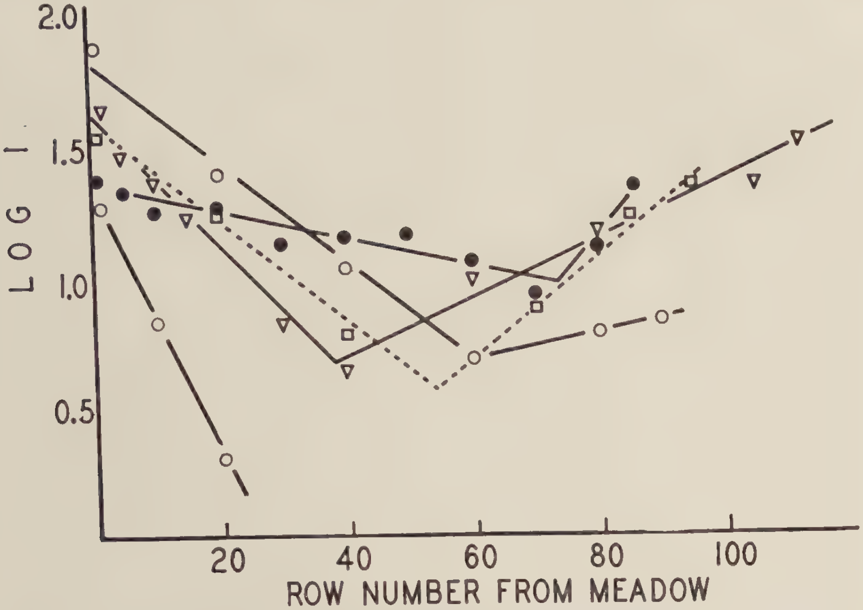

FIG. 3. The variation of the percentage of plants infected, with distance into the field, in the case of yellow-dwarf virus spreading into potato fields.

if the second basic assumption be valid, and the amount of infection occurring in the area under observation should vary directly with the time, provided the time interval is short enough so that the influence of a new brood is not of importance. The validity of the assumption involved in equation (2) was tested in a set of experiments where areas in lettuce fields containing

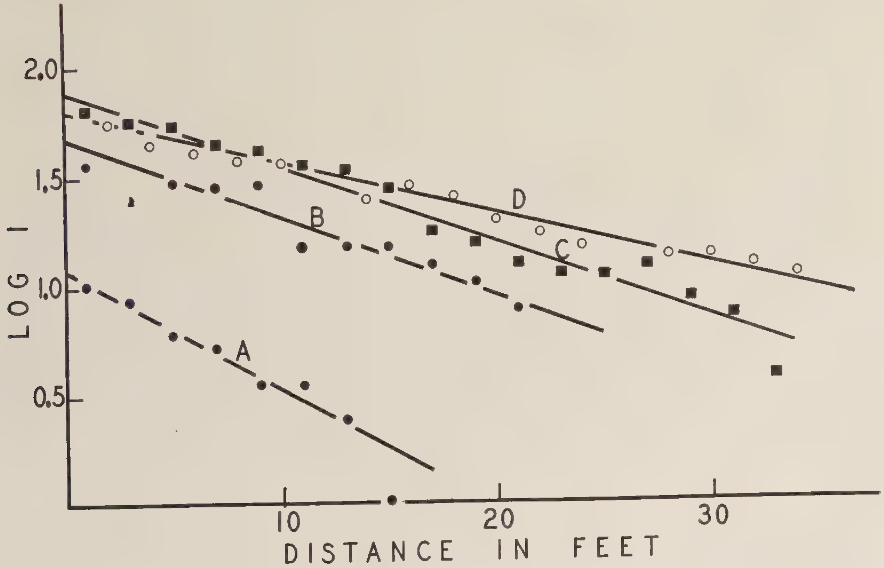

FIG. 4. The variation of the percentage of plants infected with distance into the field in the case of aster-yellows virus spreading into endive beds.

aster-yellows leaf hoppers were enclosed in a manner that precluded any substantial insect invasion. The data for the time increase in infection in the enclosure are plotted against the time in curve A of figure 5, and we see the justification for our second basic assumption.

In keeping with this line of thought, a further set of two experiments was conducted where observations were made on the time rate of infection in a fixed area to which insects had free access. The data, averaged for the two experiments, are plotted in curve B of figure 5. The amount of infection occurring in this case is not a linear function of time, but rather varies somewhat in the manner demanded by equation (6). A concurrence in the demand of equation (7) is shown in figure 6 where log I is plotted against t + log t, with the resulting straight line.

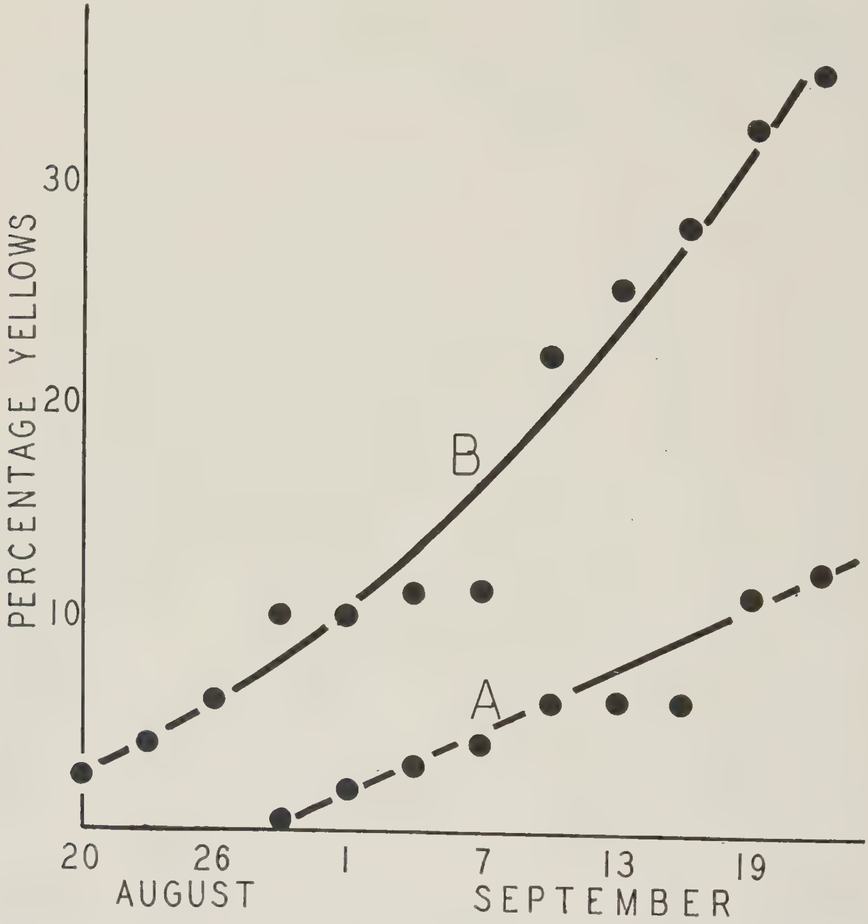

FIG. 5. The time variation of the percentage of plants infected in fixed areas in lettuce beds in the case of the spread of aster-yellows virus. Cure A is for the case where the invasion of the area by the leaf hoppers was restricted; curve B is for the case where there was no restriction imposed on the invasion of the area by the insects.

In brief, then, a differential equation describing the dissemination of viruses of the yellows type under field conditions has been derived, it has been integrated, and data obtained in a few experiments satisfy two demands of the integrated form of the equation. As has been pointed out, at the present time one is not in position to give values for the constants in the equation, and indeed each different experiment will yield different constants. In reality the full usefulness of this treatment may come when sufficient experimental work has been conducted so that one may obtain the most probable value for each of the three constants in the case of each species of insect and each crop, in which event much of the guess work in connection with the placement of field plots relative to possible insect reservoirs as a means of control of the disease will have been removed.

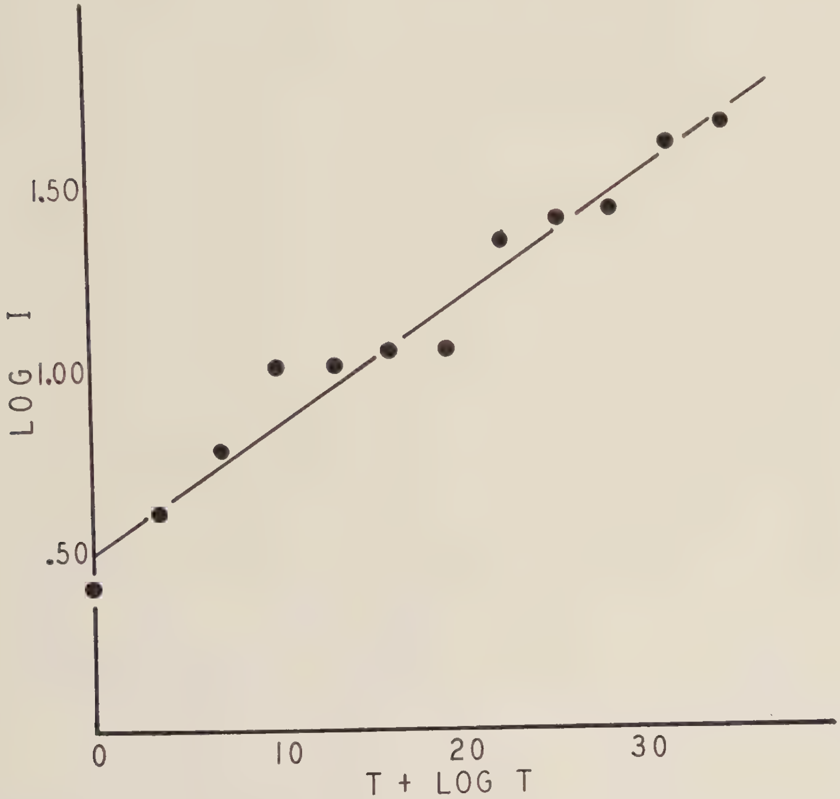

FIG. 6. The log of the percentage infection is plotted against the time + log time in a fixed area of a lettuce bed in the case where the invasion of the bed by the leaf hoppers was not restricted.

SUMMARY

A differential equation

$$\frac{\delta I}{\delta t} - \frac{I}{t} = K \frac{\delta^2 I}{\delta x^2}$$

where $I = \%$ infection
$t = \text{time}$
$x = \text{distance into field}$
$K = \text{constant}$

has been developed that describes the spread of virus diseases of the yellows type under field conditions. The assumptions involved in the derivation of this equation were that the movements of the insects involved is random, that the number of plants that become infected is proportional to the number of plants that have been fed upon, and that spread from plant to plant within the field is of small importance.

The integrated form of the equation is

$$I = Kt^{st-\sqrt{s}\,x}$$

where s and $\sqrt{s}$ are constants of integration. The boundary conditions that were set up were that the insect population in the newly plowed field was negligible, that the insect reservoir was not substantially depleted during the course of the experiment, and that the effects at the ends of the fields be neglected.

Several data obtained under field conditions satisfy two of the demands made by the integrated form of the equation.

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CLAVICEPS YANAGAWAENSIS IN IMPORTED SEED OF JAPANESE LAWN GRASS

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(Accepted for publication February 14, 1942)

In 1939 the late Dr. A. J. Pieters received a sample of seed of Japanese lawn grass, *Zoysia japonica* Steud., from Japan that contained a high percentage of sclerotia of *Claviceps yanagawaensis* Togashi (Fig. 1). The sample was tested for viability in the seed laboratory. In the first germination test, the nontreated seed was placed in moist blotters and incubated at alternating temperatures of 20–35° C. Only 36 per cent of the seed germinated. Two other samples were treated with a 75 per cent solution of sulphuric acid, the one (Lot 1) for 20 minutes and the other (Lot 2) for 30 minutes. These treatments both improved the germination very significantly. Lot 1 germinated 58.0 per cent and lot 2 germinated 58.5 per cent.[1]

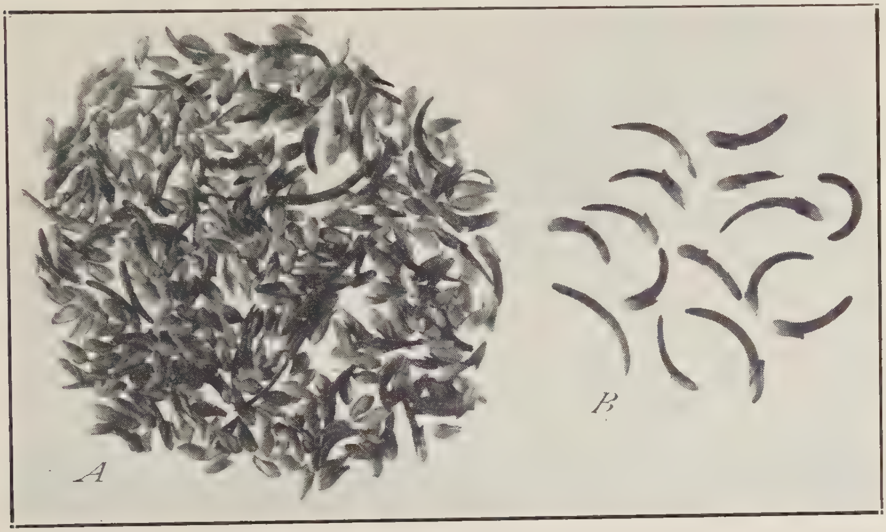

FIG. 1. A. Portion of seed sample of *Zoysia japonica* containing ergot sclerotia. B. Sclerotia of *Claviceps yanagawaensis*. ×1½.

It was observed that the sulphuric acid treatment had so charred the ergot sclerotia as to suggest their having been killed. To determine this, 3 sets of 25 sclerotia from each of lot 1 and lot 2, treated, and from lot 3, nontreated, were placed on the surface of sterilized soil in 2-in. pots. The three 2-in. pots of each set, containing sclerotia from lots 1, 2, and 3, respectively, were sunk in soil in separate 6-in. pots. The three 6-in. pots then were sunk to their tops in soil out of doors, covered with inverted clay saucers, and banked with soil to prevent excessive drying. With such an arrangement there were 25 sclerotia from each lot in each 6-inch pot under

[1] The writer is indebted to Albina F. Musil for making these germination tests.

identical conditions, and there were three replications, making 75 sclerotia from each lot included in this trial.

The sclerotia were placed as described above and the pots sunk out of

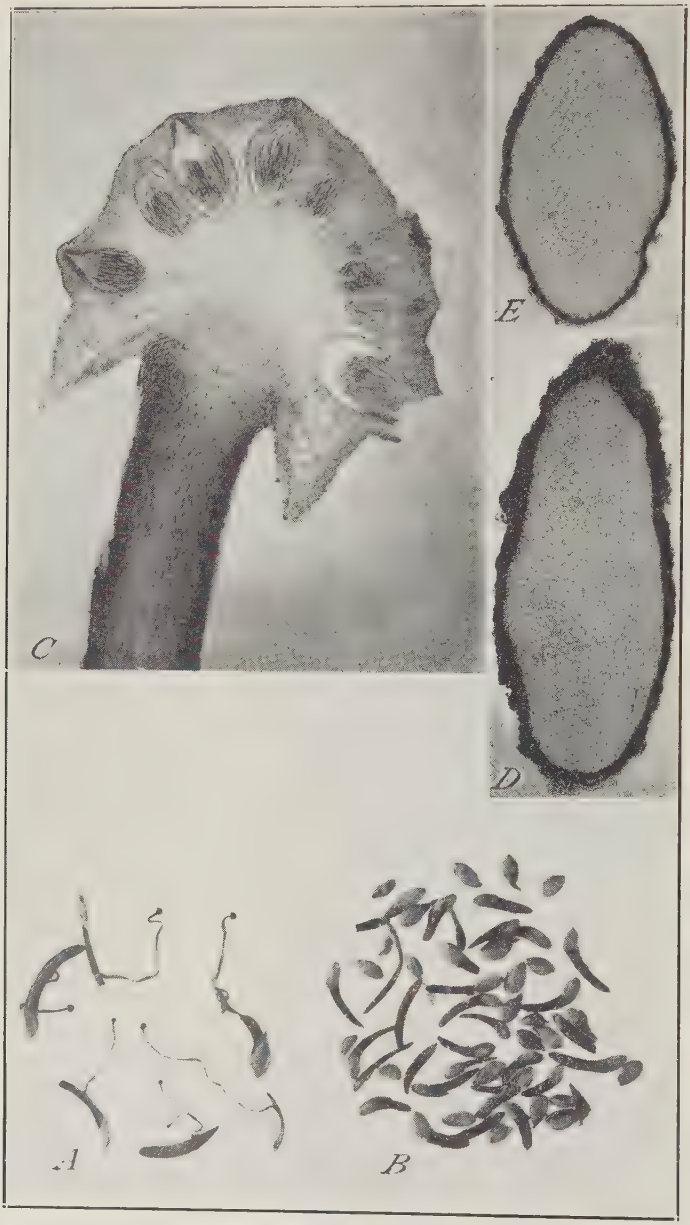

FIG. 2. Sclerotia and stromatic head of *Claviceps yanagawaensis:* A. Germinating sclerotia. ×2. B. Portion of seed sample treated 20 minutes in a 75 per cent solution of sulphuric acid showing charred sclerotia. ×2. C. Section through head and upper portion of stipe showing perithecia embedded in the head. ×75. D. and E. Cross sections of sclerotia, showing flattening. ×40.

doors on November 30, 1939, and were left undisturbed until the following spring. Counts of germinated sclerotia were made May 17, 1940. None of the treated sclerotia had germinated, while 9, 14, and 13 sclerotia, respectively, had germinated (Fig. 2, A) in the 3 nontreated lots. In other words, almost 50 per cent of the nontreated ones germinated, while none of the treated sclerotia were found to be viable.

The following year, using the same source of ergot, an attempt was made to determine the minimum treatment necessary to prevent germination of the sclerotia. The sclerotia were treated from 1 up to 30 minutes with concentrated sulphuric acid and were placed out of doors, as before, along with nontreated sclerotia. Neither the treated nor nontreated sclerotia germinated the following spring however, which indicated that they were comparatively short-lived.

THE FUNGUS

The sclerotia were 0.25 to 1.5 mm. long and 0.5 to 1 mm. wide, flattened (Fig. 2, D and E), usually slightly curved, and, for the most part, grayish-violet. Some of the sclerotia had a yellowish-green gloss over the surface that made them appear lighter in color. It was rather striking to find the shiny flattened, indurated glumes of the host attached to almost all of the sclerotia (Fig. 1).

The sclerotia germinated by producing usually 1 stroma, but occasionally 2, from the apical third of the sclerotium (Fig. 2, A). Under different conditions, the number of stromata may be greater, as shown by Togashi.[2] The stipes measured 4 to 16 mm. in length and 0.3 to 0.5 mm. in width. The length for the stipes exceeded, while their width was somewhat less than the measurements given by Togashi. This difference may be due to the fact that the writer germinated the sclerotia in the dark. Similarly, when the writer germinated sclerotia of *Claviceps paspali* S. and H. in the dark, the stipes were much longer than those from sclerotia germinated in the light.

Measurements of the heads (Fig. 2, C), perithecia, asci, and ascospores fell well within the ranges given by Togashi, which were, 0.3–1.0 mm. × 0.4–1.5 mm.; 180–320 × 70–190 μ; 85–165 × 4.0–8.0 μ; and 75–135 × 1.0–2.25 μ, respectively. From the shape, size, and color of the sclerotia, and measurements of the other reproductive bodies, the ergot found in the *Zoysia japonica* seed sample from Japan agrees well with Togashi's description of *Claviceps yanagawaensis.*

Heads of *Claviceps yanagawaensis,* containing mature spores, were crushed in sterilized distilled water. This material was then used to inoculate heads of a rye hybrid that was known to be highly susceptible to *C. purpurea* (Fr.) Tul. The rye heads were dipped in the inoculum and rolled between the fingers to insure contact of the spores with the pistil. Of the thirty heads inoculated, 10 were placed in a moist chamber for 48 hours, 10 were covered with glassine bags, while the remaining 10 were left uncovered. No infection resulted.

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[2] Togashi, Kogo. New species of parasitic fungi. I. Transactions of the Sapporo Natural History Society **14**: 280–285. 1936.

SUMMARY

Sclerotia of *Claviceps yanagawaensis* Tog. were found in seed of Japanese lawn grass imported from Japan. This fungus had not previously been reported in the United States.

Treating Japanese lawn-grass seed, containing ergot sclerotia, with a 75 per cent solution of sulphuric acid from 20 to 30 minutes killed the ergot sclerotia, whereas the germination of the seed was improved. It appears from the one test made that if such contaminated seed is treated with sulphuric acid it may be distributed for planting without the danger of introducing a new grass disease into the United States.

A rye hybrid, known to be highly susceptible to *Claviceps purpurea,* failed to become infected when inoculated with mature ascospores of *C. yanagawaensis.*

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RESISTANCE OF CERTAIN POTATO VARIETIES AND SEEDLING PROGENIES TO RING ROT

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AND ROBERT V. AKELEY[4]

(Accepted for publication February 6, 1942)

INTRODUCTION

Ring rot, caused by *Phytomonas sepedonica* (Spieckermann) Magrou, is one of the major potato diseases of the United States and Canada. It was reported from Canada by Baribeau[5] in 1931 as prevalent in potato fields of the Province of Quebec. Ring rot was discovered in the United States in 1932 in a field of potatoes in Aroostook County, Maine[6] in which State it has spread rapidly. It has been reported present in 37 States, and the resultant losses in some instances have been heavy.[7]

The planting of disease-free seed potatoes and the use of sanitary methods to avoid contamination are now generally recommended for the control of ring rot. The results of attempts to follow these recommendations have been discouraging to many growers. It has been difficult to secure seed stocks that are entirely free from ring rot. A trace of disease in the seed often has been responsible for very rapid spread within a stock. Even when seemingly adequate precautions have been taken to prevent contamination, it has been difficult to control such spread within stocks of the commercial varieties in Aroostook County. Slightly infected seed potatoes are a real menace wherever susceptible varieties are grown.

The ring-rot problem could be solved, to some extent at least, by finding or breeding resistant or immune varieties. Work on this phase of the problem is under way, and some of the results thus far obtained are reported in this paper.

MATERIALS AND METHODS

As a preliminary study in breeding for resistance to ring rot, as many as possible of the existing potato varieties were tested. Fifty-four named domestic and foreign varieties and many unnamed seedling varieties, both American and foreign, were included in the test. In addition, a limited number of the progenies of 3 crosses were tested to determine the inheritance of resistance.

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[5] Baribeau, Bernard. Bacterial wilt of potatoes. Can. Pl. Dis. Surv. Rept. **11**: 49. 1931.

[6] Bonde, Reiner. A bacterial wilt and soft rot of the potato in Maine. Phytopath. **27**: 106–108. 1937.

[7] Leach, J. G. Report of the Committee to Coordinate Research on New and Unusual Potato Diseases. Amer. Pot. Jour. **17**: 81–88. 1940.

The use of pure cultures of the ring-rot organism for inoculating potato tubers has not given reliable results in studies in Maine. Likewise hypodermic inoculations have been unsatisfactory. Therefore, a rather crude and laborious but effective method of inoculation was used. Slices of infected Green Mountain tubers were rubbed on the freshly cut surfaces of healthy seed pieces. The inoculated seed pieces were planted immediately in the field. The plants were dug after frost had killed the vines, and the individual tubers were cut into slices that were observed carefully for symptoms of ring rot.

During the winter of 1939–40 inoculations of 3 to 5 seed pieces of each variety were made in the greenhouse. Approximately 10 seed pieces of each were tested again in 1940 under field conditions. The varieties that showed no infection or only a slight amount as a result of these inoculations were tested again in 1941. The experiment in 1941 included 4 plots of each variety using 5 inoculated seed pieces for each plot.

Control inoculations were made with the susceptible Green Mountain variety, one lot for every 5 variety plots. Symptoms of ring rot are generally quite pronounced in both vines and tubers of the Green Mountain.

RESULTS

Varietal Reactions to Ring Rot. The data obtained from testing different varieties for resistance to ring rot are summarized in table 1. It may be noted that most of the 54 named foreign and domestic varieties used in this experiment were ring-rot susceptible when inoculated by the described methods. Only two of them appeared to be highly resistant.

Friso, an apparently resistant one, has been inoculated 10 times in separate experiments, and to date no infected tubers have been found. Since all 20 separate control lots of similarly inoculated Green Mountain seed pieces produced the disease in high amounts, the indication is that the Friso variety is highly resistant. This variety was obtained from the Wageningen Experiment Station through the Netherlands Legation, and is said to be resistant to leaf roll also. A British variety, President, is apparently resistant. It has been tested for the past 3 years, and no symptoms of the disease have been observed. Four selections from the cross President × Katahdin, likewise, escaped infection, also one from the cross 41956 × Earlaine. Another, from Earlaine × 43055, showed only 4 per cent ring rot.

Parent and Progeny Reactions. The data for the reactions to ring rot of Green Mountain controls, of parents used in crosses, and 3 progenies are given in table 2.

Green Mountain was used as a control because of its extreme susceptibility. President, one of the few resistant varieties, has been used quite extensively as a female parent, but, so far, has not produced enough viable pollen to make it useful as a male parent. Katahdin, a susceptible variety,

TABLE 1.—*Reaction to ring rot of American, foreign, and seedling potato varieties. Tested in Maine for several years*

Variety	Seed pieces inoculated	Plants infected
	Number	*Per cent*
Ackersegen	30	77
American Giant	20	75
Albion	10	30
Arran Cairn	10	90
Arran Consul	40	50
Arran Scout	10	90
Arran Victory	20	40
Bally Doon	56	70
Bevelander	20	50
Burbank	20	75
Charles Downing	10	60
Chippewa	20	90
Columbia Russet	28	64
Donard	20	40
Earlaine	10	40
Earlaine No. 2	19	95
Early Blue	15	67
Early Ohio	35	49
Early Rose	10	90
Ekishirazu	3	100
Flava	10	40
Friso	50	0
Garnet Chile	2	100
Gold Adler	10	30
Golden	10	50
Golden Wonder	18	78
Green Mountain (control)	100	90
Harmony Beauty	20	75
Houma	20	75
Irish Cobbler	20	90
Katahdin	40	55
Kerr's Pink	20	30
Mesaba	28	68
Noordeling	10	70
Nordeland	10	50
Norkota	20	80
Parnassia	30	70
Pennigan	10	40
Perfect Peach Blow	18	61
Pontiac	19	63
President	48	0
Prolific (Brown Beauty)	10	60
Richter's Jubel	30	67
Russet Burbank	15	87
Russet Rural	20	85
Sebago	20	55
Sequoia	20	90
Shamrock	30	60
Spaulding Rose	25	80

TABLE 1.—(*Continued*)

Variety	Seed pieces inoculated	Plants infected
	Number	*Per cent*
Triumph (from Maine)	28	54
Triumph Nebraska Strain 1	20	30
Triumph Nebraska Strain 2	20	50
Triumph Nebraska Strain 3	20	30
Triumph Nebraska Strain 4	20	40
Triumph Nebraska Strain 5	20	100
Up-To-Date	31	58
Warba	25	80
White Gold	20	45
White Rose	10	70
Ten unnamed varieties secured from McGill and Smith, Ayr, Scotland	175	40–100[a]
Six unnamed varieties secured from U. S. S. R. Institute of Plant Breeding	105	50–80[a]
Five South American varieties from collections made by McMillan and Erlanson	102	50–90[a]
Eight blight-resistant selections from true seed sent by K. O. Müller, Berlin-Dahlem, Germany	80	10–60[a]
Twenty-three blight-resistant unnamed seedling varieties from Donald Reddick, Cornell University, Ithaca, N. Y.	470	30–80[a]
Six unnamed selected seedling varieties from the cross S. 41956 × Earlaine	110	0–60[a,b]
Two unnamed seedling varieties from cross Chippewa × Katahdin	20	40–70[a]
Four unnamed seedling varieties from cross President × Katahdin	44	0
One unnamed seedling variety from cross Earlaine × S. 43055	25	4

[a] Percentage range for the various seedlings in the group.
[b] One seedling contracted no disease in 17 inoculated plants.

has many characteristics that make it a desirable parent. It is highly self-fertile, is immune from virus A in the field, and produces relatively large yields of tubers with good market quality. Seedling variety 336–123 was selected from the cross President × Katahdin because of its resistance to late blight. It is susceptible to ring rot. Seedling variety 47156 was selected because of the good cooking quality and attractiveness of its tubers. It is susceptible to ring rot. Seedling variety 3895–13 was selected from a cross the seed of which came from K. O. Müller, Berlin-Dahlem, Germany. It is resistant to late blight, but, at present, its reaction to ring rot is not known. Earlaine, another susceptible variety, is early and produces viable pollen under a wide range of environmental conditions.

Nearly half of the seedling varieties from the resistant × susceptible cross President × Katahdin escaped infection. It is improbable that these were all chance escapes, since none of the Green Mountain lots were free

TABLE 2.—*Reactions to ring rot of Green Mountain checks, parents of crosses, and three progenies*

Variety or cross	Check and parent lots tested	Seedling[a] varieties	Percentage of lots in each class of infection[b]						Total percentage of lots showing infection
			0	1	2	3	4	5	
	Number	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Green Mountain	128		....	....	4.6	13.3	33.6	48.5	100
President	12		100.0	....					0
Katahdin	20		....	....	20.0	40.0	20.0	20.0	100
336–123	20		....	....	10.0	15.0	25.0	50.0	100
47156	20		....	....	15.0	40.0	20.0	25.0	100
President × Katahdin		92	47.8	14.0	8.7	13.0	12.0	4.3	52
336–123 × 47156		20	5.0	5.0	15.0	40.0	20.0	15.0	95
3895–13 × Earlaine		22	4.0	9.0	36.0	32.0	14.0	5.0	96

[a] Test consisted of 4 lots of each seedling variety with 5 hills per lot.

[b] *Class of infection* *Infected plants grown from inoculated seed pieces*
0 = 0 per cent
1 = 1– 20 " "
2 = 21– 40 " "
3 = 41– 60 " "
4 = 61– 80 " "
5 = 81–100 " "

from the disease, and nearly half of the latter were found in the class showing 81 to 100 per cent of their tubers infected. It is evident that the President variety is heterozygous for resistance and that this character was transmitted to a relatively large percentage of the first generation progeny of the cross with the susceptible Katahdin. The data for the other two progenies, 336–123 × 47156 and 3895–13 × Earlaine, indicate that various degrees of resistance and susceptibility can be obtained from crosses between two susceptible varieties and that some of the segregates of the 336–123 × 47156 cross are more resistant than either parent.

DISCUSSION

Resistance to ring rot has been found in relatively few varieties, but it is interesting to note that it has been obtained from rather widely separated sources—Friso from the Netherlands, President from the British Empire, and a number of unrelated seedling varieties produced in the United States.

The resistant variety Friso has not yet been used as a parent because in both field and greenhouse tests its buds are abscissed and no viable pollen is produced. Attempts are being continued to find a set of environmental conditions under which it will produce flowers and pollen. President is apparently resistant, but neither Friso nor President is promising from the commercial standpoint. The most promising variety so far obtained is the selection from the cross Earlaine × 43055. This variety matures early, is resistant to virus A in the field, and produces a fair yield of tubers of excellent type in Maine. It is self-fertile and, if it continues to show the degree of resistance to ring rot indicated by the tests reported here, it should make an exceptional parent. It could be grown to advantage as a commercial crop in any section where earliness and resistance to ring rot are important factors in potato production.

Degrees of resistance and susceptibility were evident throughout the tests. For example, Green Mountain and Katahdin were both susceptible, but the Green Mountain tubers seemed to be more easily infected than those of the Katahdin. If the chi-square test for goodness of fit is applied to the data for these two varieties, using the numbers found in the various classes of infection instead of the percentages as given in table 2, there is not one chance in a hundred that these two varieties reacted alike. If resistant segregates can be obtained from crosses of 2 susceptible varieties and if nearly 50 per cent of the seedlings of a cross between a resistant and a susceptible variety are resistant, it should not be very difficult to produce varieties combining resistance with other characters of commercial importance.

SUMMARY

Ring rot, caused by *Phytomonas sepedonica* (Spieckermann) Magrou, is a major potato disease and in many instances is causing large losses to potato growers. Ring rot has spread rapidly and the presence of even a mere trace of the disease is a menace to the successful production of potatoes of susceptible varieties. The availability of resistant or immune varieties would help greatly to control ring rot.

Fifty-four named foreign and American varieties and a number of unnamed seedling varieties were tested for resistance to ring rot. All but two of the named varieties were susceptible. These two were a variety from Holland named Friso and the British variety President.

An unnamed seedling variety from the progeny of a cross S. 41956 × Earlaine and another from a cross Earlaine × 43055 have been resistant. The latter has attractive tubers and other desirable commercial qualities.

Ninety-two selections from the progeny of a cross between the resistant variety President and the susceptible variety Katahdin were tested. Nearly half of these selections were resistant, and the others varied in degree of susceptibility. A few selections from a cross between 2 susceptible varieties were more resistant than either parent.

Judging from the limited data reported in the paper it should not be very difficult to produce varieties combining resistance to ring rot with other characters of commercial importance.

A STUDY OF SPREADERS FOR USE ON HOPS[1] IN THE FIELD CONTROL OF DOWNY MILDEW

G. R. HOERNER

(Accepted for publication January 22, 1942)

The germinating zoospores of the hop downy mildew fungus, (*Pseudoperonospora humuli* (Miy. and Tak.) Wils.) infect all aerial portions of hop plants by entering the host tissues, primarily through the stomata, which are most numerous on the under sides of the leaves.

Early-season infection of the leaves of the rapidly developing shoots often progresses upwards from the bases of the vines toward the tips, due, in part at least, to wind dissemination of the zoosporangia. Infected leaves occurring high up on the vines constitute a greater menace than do those nearer the ground. Because of their location they serve, throughout the growing season, as important sources of infection of other vital parts of the vines.

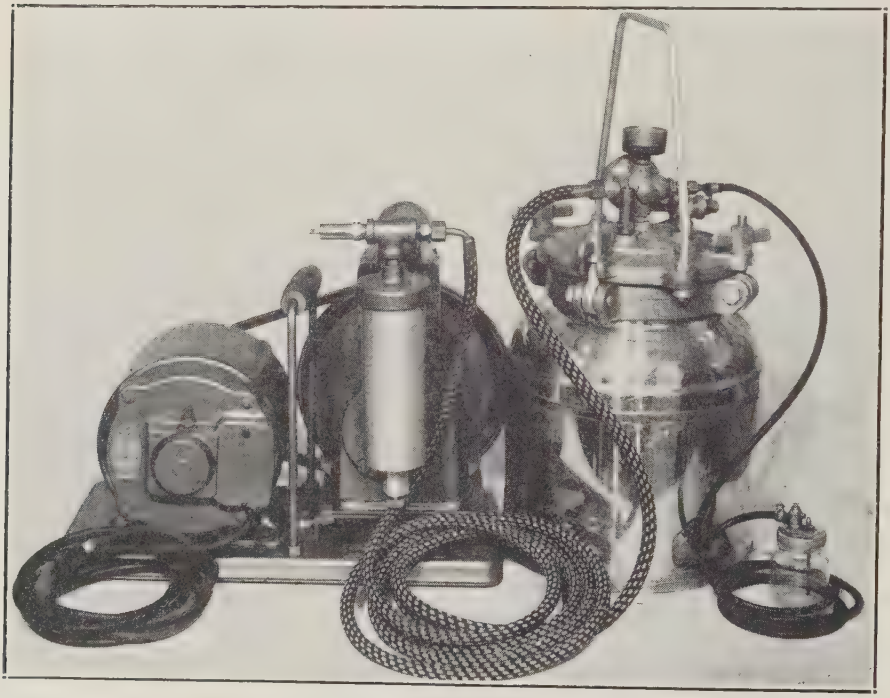

FIG. 1. Compressed-air laboratory sprayer.

USE OF A SPREADER DESIRABLE

In applying liquid sprays for the control of the disease, it is highly desirable that a maximum area of the under sides of the leaves be covered by the protectant fungicide employed.

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[1] Published as Technical Paper No. 395 with the approval of the Director, Oregon Agricultural Experiment Station.

While some protection is doubtless afforded by fungicidal applications to the upper leaf surfaces, which are relatively easy to wet, particular attention should be directed toward securing a thorough and uniform coverage of the under sides of the leaves, which is not ordinarily accomplished unless a spreader is used.

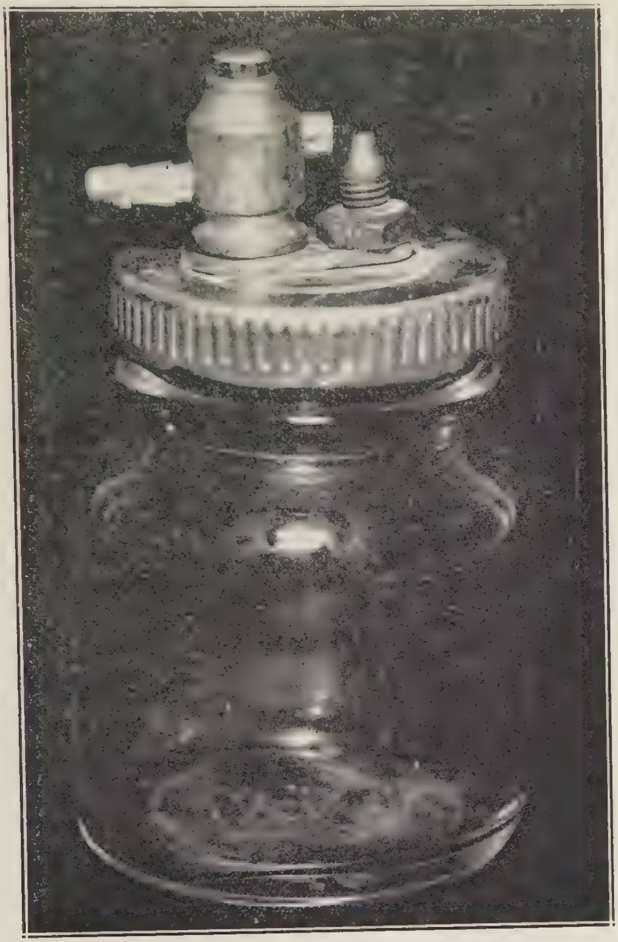

FIG. 2. Small sprayer used with air compressor.

The under sides of hop leaves are particularly difficult to wet, owing to the presence of numerous hairs and resin glands.

Numerous materials have been tested for their wetting and spreading capacity over a period of several years to determine what materials are most effective for the purpose and which are most readily available at minimum cost.

METHODS AND MATERIALS

The concentrations of all materials were reduced to the lowest points necessary to effect satisfactory coverage of the under sides of excised hop leaves in the laboratory. For this purpose a manually operated atomizer was first employed. Later in the course of the study a more satisfactory method of application was devised by employing the equipment shown (Fig. 1). It consists of a small air compressor, equipped with an oil and air filter, operated by a $\frac{1}{4}$-hp. electric motor. The air line from the compressor leads to a pressure tank equipped with a gauge that can be set at any desired point up to 50 lb. per sq. in. The use of the tank avoids surging of the air flowing from the compressor to the sprayer. The small sprayer air line is attached to the pressure tank. The sprayer nozzle, regulating the pattern of the spray, is adjustable (Fig. 2). Various materials used can thus be compared under comparable conditions.

TABLE 1.—*Approximate amounts of spreader needed for 100 gallons of water*

Material	Lowest effective dilution	Material	Lowest effective dilution
Dry	*Amount (Pounds)*	*Liquid*	*Amount (Quarts, etc.)*
Glue, flakes	2	Raylig	6 quarts
Glutin flour-baking soda	2	Skim milk-lime	5 "
Neomerpin SS	2	New Lethane Spreader	2 "
Vatsol OS	2	S.-E.C Oil	2 "
Vatsol OTC	2	Tergitol Penetrant No. 08	1.5 "
Waste sulphite, powder	2	Areskap (Aresco)	1 "
XXX Spreader	2	Ortho (Del Monte) Spreader	1 "
Actin Wetting Compound	1	Spread-ol (Silmo No. 611)	1 "
Fluxit No. 1	1	Lethane Spreader (S51)	1 pint
Hydralene	1	Liquid-soap spreader	1 "
Kayso	1	Rosin-soap spreader	1 "
Skim-milk powder	1	Santomerse	12 ounces
Felbro Whale Oil Soap	0.5	Tergitol Penetrant No. 7	8 "
Fluxit No. 2	0.5	Grasselli Spreader (SS–3)	4 "
Lethalate	0.5	Tergitol Penetrant No. 4	4 "
Swift's Tar Soap, flakes	0.5		
Blood albumin-baking soda	0.25		
Gamboge	0.25		
Saponin	0.25		
Casein-lime	0.125		
Casein-ammonia	0.0625		

Spreaders which appeared promising were then applied to the leaves of hop plants growing in the greenhouse to observe possible injury, and those that seemed safe were finally applied, in certain instances, under field conditions by means of power sprayers of various mechanical designs at various pressures with a variety of nozzles. The age and condition of the vines, the equipment and pressures employed, as well as the fungicides to which the spreaders were added, all, affected in some degree, the final field formulae.

The data presented in this paper indicate the comparative effectiveness of the materials listed below when applied alone to the under sides of hop leaves in laboratory or greenhouse only.

In the following table the amounts given under the heading "Lowest effective dilution" represent the approximate amounts of spreader needed for one hundred gallons of water.

The rosin-soap spreaders appeared particularly promising. At least 6 different formulae, which varied in the amount of rosin, caustic potash or soda, fish or corn oil, were compared. The final field formula adopted consists of a stock solution prepared from: 25 lb. rosin, 6 lb. caustic potash, and 25 gal. water. A minimum of 1 pint of stock solution is recommended with 100 gal. of any fungicide employed in the field.[2]

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[2] Hoerner, G. R. A Rosin-potash spreader for spraying hops for downy mildew control. Ore. Stat. Cir. Inf. 236, 2 pp. 1941.

METHOD OF OBTAINING PURE CULTURES OF CORTICIUM STEVENSII FROM SCLEROTIA

E. C. TIMS AND FRANCES BONNER

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Considerable difficulty often has been encountered in obtaining pure cultures of *Corticium stevensii* (Burt) from sclerotia at Louisiana State University. Other workers[1] also have had trouble obtaining pure cultures of the thread-blight organism by plating sclerotia from infected twigs. In connection with studies of fig leaf blights in Louisiana, attempts were made to develop a satisfactory method for obtaining pure cultures from thread-blight sclerotia.

Fig twigs of the current season's growth with numerous sclerotia of the thread-blight fungus on the surface were collected from several locations in southern Louisiana, and infected tung twigs were obtained from groves near Bogalusa, Louisiana. The sclerotia were removed from the twigs, sterilized or otherwise treated, and plated on water agar. Several treatment methods were used, including (1) rinsing in sterile water, (2) dipping in 95 per cent ethyl alcohol and flaming, (3) immersing in 1–1000 $HgCl_2$ solution in 50 per cent alcohol and rinsing in sterile water, and (4) immersing in the $HgCl_2$-alcohol solution, then dipping in a saturated aqueous solution of calcium hypochlorite. The sclerotia were plated at intervals from October 1940 through April 1941, and the desired fungus (*Corticium stevensii*) was isolated from each lot.

The plates were examined after 4 to 7 days incubation at about 24° C. in most cases; some were kept at room temperature. The colonies of *Corticium stevensii* were identified by microscopic examination. These forms have a characteristic type of branching and septation. Representative cultures were made from the different lots of sclerotia and compared with known cultures of *C. stevensii.*

During the course of the experiments more than 1500 sclerotia were plated, most of them from fig, but several hundred were from tung twigs. The percentages of sclerotia from which *Corticium* grew varied from about 35 to 94, but the numbers of sclerotia free of contamination were much lower. Some of the sclerotia had various faster growing fungi developing from them, which made it impossible to transfer the slower growing *C. stevensii.*

The results varied considerably with the different lots of sclerotia plated. One typical test is given here to indicate the results obtained. Sclerotia from tung and fig branches were subjected to 3 types of treatment. Each lot consisted of 50 sclerotia. Some lots were treated with the $HgCl_2$-alcohol solution for 2 minutes and rinsed in sterile water; others were dipped in the calcium hypochlorite solution after receiving the $HgCl_2$-alcohol treatment. Two lots of sclerotia were dipped in alcohol and then flamed. All lots were plated on water agar after being treated. The results obtained after 6 days' incubation at about 25° C. are given in table 1. Relatively large numbers of *Corticium stevensii* were obtained from most lots of sclerotia, the smallest numbers being found on those from tung twigs. The numbers of this slow-growing form that could be transferred varied from 2 to 40, but most of the lots had comparatively large numbers of colonies from which *C. stevensii* was successfully transferred.

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[1] Wolf, F. A., and W. J. Bach. The thread blight disease caused by *Corticium koleroga* (Cooke) John, on citrus and pomaceous fruits. Phytopath. **17**: 689–709. 1927.

TABLE 1.—*Results obtained from plating thread-blight sclerotia from tung and fig branches after various treatments*

Host	Locality	Number of sclerotia plated	Treatment	Number giving growth of *Corticium stevensii*	Number from which *C. stevensii* could be obtained in pure culture
Tung	Bogalusa	50	[a]	22	7[d]
Tung	Bogalusa	50	[c]	22	2[d]
Fig	Union	50	[a]	46	40
Fig	Union	50	[c]	38	35
Fig	Union	50	[a]	41	24
Fig	Union	50	[a]	43	22
Fig	Union	50	[a]	33	19
Fig	Union	50	[b]	35	31

[a] = $HgCl_2$–50 per cent alcohol, 2 minutes.
[b] = $HgCl_2$–50 per cent alcohol, $CaOCl_2$.
[c] = Flamed in alcohol.
[d] = Much larger numbers of *C. stevensii* were obtained from later platings of sclerotia from tung.

This brief summary of studies carried on over a period of one year shows that pure cultures of *Corticium stevensii* can easily be obtained from sclerotia not more than 1 year old if they are soaked in a mixture of $HgCl_2$ 1–1000 in 50 per cent ethyl alcohol for 2 minutes, washed in sterile water, and plated on water agar. Other treatments also gave some good results.

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REPORT OF THE TWENTY-SIXTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAL PHYTO-PATHOLOGICAL SOCIETY

The 26th annual meeting of the Pacific Division of The American Phytopathological Society was held at the University of Utah, Salt Lake City, June 17–19, 1942, in conjunction with the annual meeting of the Pacific Division of the American Association for the Advancement of Science. Members were present from Arizona, California, Idaho, Utah, Wyoming, Washington, D. C., and Brazil. Twenty-two members attended the scientific sessions, 17 the dinner meeting, and 10 the field trip.

Sixteen volunteered papers were presented at 3 half-day sessions. At one half-day symposium on "Breeding for resistance to plant diseases," under the chairmanship of H. Loran Blood, and in cooperation with the Western Section of the American Society for Horticultural Science, 8 invitation papers were presented.

Officers of the society for the ensuing year are as follows: President, L. D. Leach, University of California, Davis; Vice-President, B. L. Richards, Utah Agricultural College, Logan; Secretary-Treasurer, C. E. Yarwood, University of California, Berkeley; and Councilor, Glenn A. Huber, Western Washington Experiment Station, Puyallup.

The next annual meeting is scheduled to convene in Corvallis, Oregon, in June, 1943. Titles and abstracts of papers presented at the meeting follow.

C. E. Yarwood,
Secretary-Treasurer.

Control of Crown Gall of Peach in the Nursery. Ark, P. A. Experimental data obtained in 1941 indicate that when peach pits are dusted with a slightly water-soluble chemical mixed with an inert insoluble material, such as Celite 500, there is a marked decrease in incidence of crown gall on the resulting peach stock. The following chemicals mixed at the rate of 15 g. per 450 g. of Celite 500 and applied to the thoroughly washed peach pits were not effective in preventing crown gall: calomel, copper carbonate, copper stearate, and thymol, showing 85.3, 94.3, 98.2, and 100 per cent disease, against 99.1 per cent in the check and 96.2 in Celite 500 dust. In contrast, Ceresan, mercuric cyanide, and mercuric iodide dusts showed only 3.8, 13.9, and 4.3 per cent disease. Acidification of the soil with agricultural sulphur, reducing the original pH of 7–8.5 to pH 5–5.5, caused a decrease in the amount of crown gall from 20 to 30 per cent. An application of 2000 lb. of sulphur per acre caused yellowing and stunting of the plants, while applications of 500 and 1000 lb. showed no injurious effects upon the peach stock.

Root Scab of Carrot Caused by Phytomonas carotae. Ark, P. A. and M. W. Gardner. A scab of carrot roots, serious in California, and probably identical with one described by Ramsey and Wiant in 1941, results from direct root infection by *Phytomonas carotae*, cause of the bacterial blight of the leaves and umbels, described by Kendrick in 1934. Root infection abounds in irrigated fields cropped repeatedly to carrots. Very young lesions are small, rather inconspicuous, brown or maroon spots, often numerous. Early infection may result in sharply sunken constrictions of the root or large, rough, sunken cankers. Later infection results in laterally elongated, brown or black, rough, scabby lesions, often protruding because of the tremendous masses of the bacteria that ooze out and embed particles of soil. Particularly objectionable are the occasional fairly normal-appearing roots with healed-over internal pockets of blackened, infected tissue. Secondary fungi may invade infected roots. The bacteria may persist in the soil in the field as long as 6 months. Diseased roots culled out at harvest should not be left in the field. To prevent the introduction of the pathogen into noninfested soil, the seed may be treated for 10 minutes in a water bath at 50–52° C.

Longevity of Curly-top Virus in Dried Tissue of Sugar Beet. Bennett, C. W. In July, 1934, small sugar-beet plants affected by curly top were dried at room temperature, divided into 4 lots, and each lot stored in a separate container under laboratory conditions. Lot 1 was exposed to the humidity of laboratory air throughout the test period. Lot 2 was placed over calcium chloride in an air-tight container. Lot 3 was placed over calcium chloride in an air-tight container and the air was replaced by hydrogen for the first year; during the remaining period of test, air was admitted. Lot 4 was stored in an air-tight container and the air was replaced by hydrogen for the first year; during the remaining period of test, air was admitted. Tests of active virus content were made at the beginning of the experiment and annually thereafter, except in 1940, for 8 years. Twenty seedling beets were inoculated from each lot in each test. The plants infected in tests of successive years of test from each lot were as follows: Lot 1; 3, 3, 11, 0, 0, 0, 0, 0. Lot 2; 2, 2, 8, 3, 7, 0, 2, 0. Lot 3; 5, 1, 18, 1, 2, 2, 3, 2. Lot 4; 3, 0, 12, 0, 0, 0, 0, 0. These results show that when the virus is kept in a thoroughly dry condition it remains active for a period of at least 8 years.

Statistical Studies of Distribution of Psorosis Cases in Citrus Orchards. Bitancourt, A. A. and H. S. Fawcett. The majority of the cases of psorosis are apparently due to the utilization of buds from diseased parent trees and the different operations from the collection of bud sticks to the final planting in the orchard usually afford ample opportunities for a thorough randomization. However the comparison of the mean number of cases of psorosis in the 4 trees adjacent to diseased trees and adjacent to healthy trees has shown highly significant differences in 7 orchards, a significant difference in 1 and no significance in 3 orchards. In those 11 cases there were more diseased trees adjacent to diseased trees than to healthy ones. In an orchard showing very low percentage of psorosis, a nonsignificant difference was found in favor of the healthy trees. The same analysis made independently for the 2 adjacent trees along rows and the 2 adjacent ones along arrays has shown significant differences in both directions in 5 orchards, along rows only in 1 and along arrays only in 2. Systematic arrangement of diseased trees along rows or arrays at the time of planting is, therefore, indicated in the last 3 cases, while transmission from diseased to healthy trees is the most probable explanation for the 5 others.

Coryneum Blight of Stone Fruits. Blodgett, Earle C. Although present for many years and generally serious in northern Idaho, Coryneum blight of stone fruits has increased in recent years and become serious in the drier (southern) part of the State. The disease, usually confined to peach and apricot has occurred in extreme severity in commercial sweet and sour cherry orchards, and in smaller plantings of Italian and Agen (French prune). Inoculation studies using conidia of *Coryneum beijerinckii* from peach apricot, pin cherry [*Prunus emarginata* (Dougl.) Walp.] Cherry, Italian prune, and almond produced abundant leaf spots and twig lesions on potted trees in the greenhouse of Slappey Peach, Moorpark Apricot, Bing and Montmorency Cherry, Italian prune, Ne Plus Ultra Almond, and on Stanwick Nectarin (in the case of isolates from pin cherry, prune and almond). Some lesions appeared within 48 hours after inoculation. The tests showed that under the conditions encountered, the largest average number of spots per leaf, about 245, occurred with the isolates from peach and Royal Ann Cherry on Bing Cherry. The isolate from pin cherry produced the most severe spotting, 162, on prunes. Apparently *Coryneum beijerinckii* isolated from various hosts is pathogenic on the commonly cultivated stone fruits.

Effect of Temperature on the Epidemiology of Sugar-beet Downy Mildew. Carsner, Eubanks, Charles Price, and Glenn E. Gillespie. Downy mildew often develops extensively on sugar beets grown for sugar in the coastal areas of California and on sugar beets grown for seed in the Willamette Valley of Oregon and along Puget Sound in Washington. Temperature appears to be the most important factor influencing the epidemiology. Germination of the spores and infection will occur at relatively low temperatures. Sporulation requires slightly higher temperatures. All development of the fungus stops at about 70° F. and infected plants that have not been too severely injured begin to recover.

Shock Effect of Certain Viruses of the Mosaic Group on Peach. Cochran, L. C. Observations and surveys in mosaic-affected peach orchards have substantiated the suggestions of growers and inspectors that severely retarded trees are initial cases of the disease. Less severely affected trees of tolerant varieties often show symptoms only during the initial year of the disease. Transmission annually for 3 years from individual severely and mildly affected orchard trees has shown symptoms to be much more acute in the respective test trees the first year after inoculation. Subsequent transmission from such test trees, after partial recovery, gave acute symptoms approximating those of the first year of infection in the first set. The ring-spot virus of stone fruits produces acute symptoms the initial year of the disease in peach but only rarely shows any symptoms the second year, in which cases the tree was not universally invaded the first year. Subsequent inoculations from symptomless carrier trees result in acute symptoms. In other hosts where the ring-spot virus causes symptoms annually, symptoms are more intense during the first year of the disease. Similar results have been obtained with two undescribed viruses causing mottling on peach. This phenomenon is regarded as shock effect correlated with the first stage of the disease.

Limitations in the Control of the Virus Diseases of Peach Through Plant Breeding. Cochran, L. C. Prior to 1931 all viruses known to infect peach affected all horticultural varieties similarly, moreover, some infected other stone fruits that could be hybridized with peach. This afforded no starting point for the plant breeder and left no alternate to control through infected tree removal. Soon after the discovery of the peach-mosaic disease it was noted that certain peach varieties could be affected with little damage. While this appeared encouraging, it was soon found that such varieties were reservoirs from which the severe form of the virus could spread. Work with vigorous sport-like shoots on severely mosaic-affected trees and with immunizing trees with a mild form of the virus has shown promise; but both host and virus stability need further investigation. The occurrence of virus strains and variance in symptom intensity within the mosaic complex has further complicated the problem. The production of a desirable immune variety is remote, and would involve a long-time, intensive breeding program. Numerous recently discovered peach viruses have appeared in the literature. Some of these, as the X disease, appear to infect and damage all peach varieties similarly, and some are too cosmopolitan in host range to offer much hope to plant breeders.

Breeding Beans for Resistance to Powdery Mildew and Rust. DUNDAS, B. By testing detached leaflets in Petri dishes the reaction of individual plants of segregating populations to the various forms of mildew and rust can be determined. It is thus possible to determine the number of relationships of the genetic factors giving resistance to these several forms. Pinto, other field beans, and some garden varieties carry a main dominant factor for resistance to 12 of the 14 forms of mildew isolated. This factor is being incorporated into new garden beans now under development. There is also a dominant factor for semi-resistance, and one causing susceptibility during 5–7 days after emergence. A number of factors for resistance to rust have been found in various garden beans. The proper combination of certain of these factors should give resistance to all 20 known forms of rust. A combination of the factors for resistance in Golden Gate Wax and Brown Kentucky Wonder 928 has been used in breeding for resistance to 4 forms of rust. Recently several new varieties have been released that are resistant to some forms of rust. Other promising lines carrying resistance to more forms of rust, mildew, and bean virus No. 1 should be forthcoming.

Perithecia of Bean Powdery Mildew. DUNDAS, B. In the fall of 1941, immature perithecia were independently found by Middleton and Yarwood on Lima beans infected with powdery mildew, in Southern California. The writer subsequently established the fact that conidia from the leaves containing perithecia always produced infection of form 1, the form most commonly found on Lima beans. Assuming these perithecia are from the same mildew which produced the conidia, the appendages showed them to belong to the genus *Microsphaera* and not to *Erysiphe*, as has been previously supposed.

Wood Alteration in Psorosis of Citrus in Relation to Tree Decline. FAWCETT, H. S., A. A. BITANCOURT, and J. M. WALLACE. In psorosis A and B, (1) the primary lesions in the wood consist of gum in and between part of the vessels, (2) secondary lesions in the wood result in irregular discoloration and necrosis. The latter usually develop after the branch or trunk is girdled by the primary lesions of the bark and wood. Suction experiments in drawing water or aqueous stains (as 0.5 per cent acid fuchsin or sufranin) through the wood indicates that the presence of the primary lesion has little effect on water passage, but that the secondary lesions, as well as a region contiguous to the visibly affected part are impervious to water passage. These regions are coextensive with those in which the I-KI test shows absence of starch. Experiments in drawing air through wood of branches showed that larger amounts of air passed through bark with psorosis lesions than through normal bark and that in normal bark air passed slowly only through the lenticels. The lack of water passage caused by formation of the secondary wood lesions appears to be the principal factor in tree decline. The alterations and discoloration of the central wood layers probably are explained by their starvation due to blocking the passage of food inward by gum in the primary wood lesions nearer the surface.

Progress in Breeding Sugar Beets for Curly-top Resistance. OWEN, F. V., ALBERT M. MURPHY and BION TOLMAN. Curly-top-resistant varieties of sugar beet have now definitely revived the sugar-beet industry in the Western States. In many tests high yields have been obtained with the resistant varieties where the susceptible European varieties failed completely. U. S. No. 1, grown commercially on a limited scale in 1934 and extensively in 1935, was the first curly-top-resistant variety released by the United States Department of Agriculture. Varieties U. S. 34, U. S. 33, and U. S. 12 soon replaced U. S. 1, but of these the high sugar-producing variety U. S. 33 is the only one that will be continued in commercial use. U. S. 22, a new variety with a higher degree of curly-top resistance, is now available to commercial growers. If given good cultural care, the U. S. 22 beets make good growth in spite of curly-top infection; but, if neglected and allowed to suffer for irrigation water, some curly-top damage may result. The advantage of having still higher curly-top resistance is obvious, and new improved selections are being propagated as rapidly as possible. The variety U. S. 1 and all other curly-top-resistant varieties so far produced have been highly self-sterile, a feature that ~~has~~ made mass selection the principal means of improvement. However, many curly-top-resistant self-fertile lines are now available to facilitate inbreeding work.

The Big Vein Disease of Lettuce in Relation to Soil Moisture. PRYOR, DEAN E. An investigation of soil moisture in relation to the big-vein disease of lettuce was made on two different infective soils, one from near Salinas, California, and the other from the Imperial Valley, California. The number of big-vein plants appearing on highly infective soil increased with the increasing moisture. A considerable number of diseased plants was present, even at moisture levels below the optimum for growth of lettuce, and a few plants had big vein when supplied with only enough water to keep them alive. The greatest disease incidence occurred in the treatments producing the largest plants. Plant weight increased with each increment of moisture used. The more vigorously growing individuals in each series seemed to be most readily affected with big vein, but the earlier a plant showed the disease the smaller was its final weight. In the Salinas soil, diseased plants seemed to be larger than healthy plants in the same pot, while the reverse was true in Imperial Valley soil. The difference was attributed to a longer growing period for plants in the Imperial Valley soil.

Morphological Similarity in Culture Between Torulopsis pulcherrima and Taphrina deformans. ROBERTS, CATHERINE. Three- to five-month-old cultures of *Torulopsis pulcherrima* and *Taphrina deformans* on potato dextrose and vegetable (beet-cucumber-carrot-potato) agar exhibited decided similarities in cellular morphology. In 2 out of 4 isolates of *Torulopsis pulcherrima* globose, thick-walled cells containing single fat globules and with attached remnants of cell walls were observed. Other cells possessed single bud-like protrusions containing 1 to 2 spores, thus partially confirming observations on sporulation made by Windisch in 1940, who interpreted the globose cell as an ascus mother cell, the protrusion as an ascus, and the attached wall remnant as the remains of an ascus following ascospore discharge. In *Taphrina deformans* similar globose, thick-walled cells containing one to many fat globules were observed, together with attached wall remnants. Bud-like protrusions also were present, some of which contained 1 to 3 ellipsoidal bodies believed to be ascospores, although their discharge and germination have not been observed. These observations, together with the similarity evident in macroscopic cultural behavior, including dissociation into pigmented and non-pigmented areas, suggest that *Taphrina deformans* and *Torulopsis pulcherrima,* at least those isolates studied, are more closely related than is indicated by their positions in the present systems of fungus classification.

Attempts at Mass Infection of California Citrus Red Scale with Bacteria. SOKOLOFF, V. P. and L. J. KLOTZ. Approximately 5×10^{12} spores of the red scale bacillus (Phytopath. 32: 187–198) were sprayed and dusted over lemon trees in 2 field trials conducted in September and December, 1941. A demonstrable increase in mortality of the scale was noted on leaves, green bark, and fruits of the sprayed-dusted trees. Density of the red scale population decreased significantly on the infected trees, parallel with the decreased percentage survival. In the December experiment a premature mortality developed among the scale throughout the 2-acre grove, on the 60th-70th day, whereupon differences between the controls and the infected trees had disappeared. The microorganism used in the experiment and some other species of the genus *Bacillus* could be isolated invariably from the prematurely dead adults and gray adults in sections of the orchard far removed from the site of the experiments. Among other organisms associated with the premature death of the scale, there were some common, apparently non-pathogenic fungi and a species of *Actinomyces.*

The Effects of Some Natural Gases Upon Plants. SOLHEIM, W. G. and RALPH W. AMES. The effects of a natural gas from the Billy Creek field, source of the gas used in Sheridan, Wyoming, and one from the gas mains of Laramie, Wyo., were studied. (The gas from the Billy Creek field was supplied through the courtesy of the Northwest States Utilities Company of Sheridan, Wyo.) The composition of the Sheridan gas, according to an analysis made by the U. S. Bureau of Mines, is as follows: carbon dioxide 0.2 per cent; oxygen 0.2 per cent; methane 97.5 per cent; ethane 0.0 per cent and nitrogen 2.1 per cent. According to an analysis made by the Kansas City Testing Laboratory of Kansas City, Missouri, the Laramie gas has the following composition: methane 71.5 per cent; ethane 22.3 per cent; carbon dioxide 0.0 per cent; oxygen 0.2 per cent; olefines 0.0 per cent and nitrogen 6.0 per cent. Tomato, potato, sunflower, castor bean, and geranium plants were in no way affected by concentrations of 4, 6, 12, 24, and 50 per cent for 24-, 48-, 72-, 96-, and 120-hour treatments. Cut carnations did not exhibit any symptoms of injury when treated with 1 and 2 per cent concentrations for 24, 48, 72, and 96 hours. When Fuchsia plants were exposed to gas concentrations of 4, 12, 24, and 50 per cent for 24, 48, 72, and 96 hours a slight browning and wilting of the petals was noted. The tests reported above were made in 1940. In 1941 further tests were made on tomatoes with gas from the Billy Creek field. These tests confirmed the original ones —no effects being observed from the gas.

Investigation of Storage Diseases of Carrots. VIRGIN, W. J. A considerable amount of carrot seed is produced in Idaho each year. The climatic conditions are such that it is necessary to store the roots through th winter and set them out in the spring, rather than leave them out in the field all winter. The grower either stores them in a pit or a potato cellar. Each year there is considerable loss in storage due to the organisms *Botrytis cinerea* and *Alternaria radicina.* At near-freezing temperature growth of these organisms is almost stopped. Consequently, the best storage is the one in which the temperature remains near freezing. It was found that pit storage was superior to cellar storage, since the temperature could be maintained near 0° C. and it did not fluctuate so much as did the cellar temperature. At temperatures above 5° C. carrot roots in storage soon produced new leaf growth. These new leaves were very susceptible to infection by *B. cinerea,* and, from the leaves, the organism soon invaded the crown and completely destroyed it, rendering the carrot useless for seed production. The other important point of entry for *B. cinerea* was the fine tip end of the tap root.

Function of Lime and Host Leaves in the Fungicidal Action of Bordeaux Mixture. YARWOOD, C. E. To give 95 per cent inhibition of germination of bean-rust urediospores on glass surfaces required a dried deposit of 3.2 mg. per sq. dm. of bluestone or 450 mg. of equal lime-Bordeaux mixture. To give 95 per cent reduction in number of uredial pustules of bean rust (=95 per cent control) in greenhouse tests under conditions of heavy artificial inoculations required 4.5 mg. bluestone but only 0.44 mg. Bordeaux mixture per sq. dm. of lower leaf surface. Therefore, to give equivalent control, 1000 times as much Bordeaux was required on glass as on leaves, more bluestone was required on leaves than on glass, bluestone was more toxic than Bordeaux on glass. Bordeaux, however, was far more fungicidal than bluestone, on leaves. The copper in bluestone spray was rapidly absorbed by bean leaves with consequent reduction in control, but the copper in Bordeaux was absorbed to a much smaller extent. These results indicate important unreported functions of lime in Bordeaux mixture; but the above principles have not as yet been confirmed with cucumber downy mildew.

Fungicide Treatment of String Supports for Control of Hop Downy Mildew. YARWOOD, C. E. In California the principal damage from hop downy mildew is due to infection of the nodes and growing points of the trained vines. This infection usually occurs during rainy periods. Because the terminal growing points and nodes are close to the strings on which the vine is trained, and since Magie has shown (Phytopath. **30**: 16, 1940) that the drip from Bordeaux-sprayed hops protected non-sprayed growth, it was thought that if the strings were impregnated with a suitable fungicide, enough of the fungicide might be scattered by splashing and dripping rain to kill the spores at the nodes and growing points. String treatments with various copper, sulphur, and organic materials were tested in 6 hop yards in 1942. The natural drip during rain from cotton strings treated with low lime-Bordeaux (7.5 per cent bluestone plus 2.5 per cent lime) when diluted to 1/20 its field strength prevented germination of the sporangia of *Pseudoperonospora humuli* in watch glasses. Vines growing on strings treated with the low lime-Bordeaux had 80 per cent fewer leaf infections, 50 per cent fewer nodal infections, and 78 per cent fewer infected basal shoots than vines on untreated strings in one hop yard.

THE WAR EMERGENCY COMMITTEE
of
THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The purpose of the War Emergency Committee is to provide for coordinated effort in research, experimentation, and extension work designed to control destructive diseases of plants. To accomplish this aim there is a general committee, comprising a number of members at large and the chairmen of regional groups that perform the same function in certain natural geographical divisions as the general committee does for the country as a whole.

Past experience indicates very clearly that it is imperative in war time to maintain an efficient plant quarantine service. New diseases and pests are very likely to be introduced and distributed because of the exchange of plant propagative materials between certain countries and within the United States. The expansion of acreage of certain crops and the consequent interchange of seed and other propagative parts of plants intensify the problem. Indeed, some new diseases already have made their appearance during this war. Furthermore, there is always the likelihood that the increase in prevalence of new parasitic strains of pathogens already present may develop on hitherto resistant varieties of crop plants; consequently it is of the utmost importance to maintain adequate plant disease survey services in order to provide for the early detection, extermination, and control of new diseases or of unusually virulent strains of old disease organisms.

Two of the best methods for preventing the indiscriminate distribution of plant pathogens are seed certification and seed treatment. For this reason, provision has been made in the organization of subcommittees for cooperative effort in improving seed certification and for obtaining new information and disseminating old information regarding fungicides used to treat seeds and other propagative parts of plants.

Because of priorities there is a shortage of certain commonly used fungicides, and the need therefore develops for substitute fungicides. A subcommittee is working hard and effectively on this problem and, in cooperation with fungicide manufacturers and with governmental agencies, is attempting to develop substitute fungicides and to determine the most economical ways of using those now available.

It is of course of the utmost importance to provide for the proper dissemination of essential information regarding plant diseases, and a subcommittee on extension activities is therefore attempting to codify important control measures and to assist in obtaining provision for proper dissemination of the information.

During World War I, a War Emergency Board of American Plant Pathologists under the chairmanship of Professor H. H. Whetzel made significant contributions to insuring the nation's food supply through the control of plant diseases. The situation with respect to foodstuffs has not yet become acute in this war, but the situation with respect to some essential plant materials already is acute, and the control of plant diseases and insect pests is therefore of paramount importance. The American Phytopathological Society, through its War Emergency Committee, is again attempting to pool the services of plant pathologists insofar as possible, in order to alleviate present situations with respect to plant diseases and to safeguard the future. The membership at a summer meeting in Toledo, June 25 and 26, discussed war problems extensively and attempted to provide insofar as possible for the solution of immediately pressing problems. It was the consensus of opinion that basic research designed to elucidate principles on which control measures must be based is even more important in war time than in peace time, in order to avoid mistakes in the present and to lay a solid foundation for future procedures.

The personnel of the Committee and its Subcommittees follows:

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C. C. Allison, Secretary of The American Phytopathological Society, Botany Department, University of Ohio, Columbus, Ohio.
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JOSEPH CHARLES ARTHUR

JOSEPH CHARLES ARTHUR
1850–1942

FRANK D. KERN

In the death of Joseph Charles Arthur at Brook, Indiana, April 30, 1942, we have lost an eminent botanist, a pioneer plant pathologist, and one of the foremost students of plant rusts in the world.

Joseph Charles Arthur was born at Lowville, New York, January 11, 1850, the son of Charles and Ann (Allen) Arthur. His earliest paternal American ancestor was his great-great-grandfather who came from England about 1745 and settled in Groton, Connecticut. The first American-born ancestor was Richard Arthur, whose wife, Hannah Bradford, was a great-grand-daughter of Governor William Bradford, one of the Mayflower pilgrims. One of the sons of this marriage was Joseph Arthur, grandfather of Joseph Charles Arthur. After the death of her husband Mrs. Richard Arthur moved from Connecticut to northern New York. This was the family abode for a considerable time. However, Charles Arthur, father of Joseph Charles, went westward when his son was about six years old and settled near Sterling, Illinois, but soon pushed on into Iowa. The family home was at Charles City, Iowa, for a number of years, and then at Spirit Lake. At Charles City they lived for a time in town and later on a farm.

Joseph Charles received his preliminary education in the country schools of Floyd County and in the high school of Charles City. The boy was christened Charles Joseph, but, since his father's name was Charles, he was called Joseph, at home. In school he was registered as C. Joseph Arthur. When the teacher called the roll the other youngsters often turned about in their seats to "see" Joseph Arthur. This was so annoying to the sensitive boy that he changed the order of his name to Joseph Charles and retained that form ever afterwards.

From Floyd County he went as a student to the newly established Iowa State College at Ames. Here, in 1872, he was graduated first on the list (alphabetically) of the first graduating class, with the degree of Bachelor of Science. In 1877 he earned there the degree of Master of Science. In 1886 he was the recipient of the first degree of Doctor of Science conferred by Cornell University in the field of plant pathology and mycology. His honorary degrees include the Doctor of Laws, University of Iowa, 1916; Doctor of Science from his alma mater in 1920; and Doctor of Science, Purdue University, 1931. He was a graduate student at Johns Hopkins and at Harvard in 1879 and at Bonn in 1896.

The ambition to be a botanist appeared very early in the life of Joseph Charles Arthur. He had it as a boy before he went to college. The development of this ambition and the persistence with which he clung to it make a most interesting story. How eminently well he succeeded has long been recognized in scientific circles everywhere. The early difficulties are worthy

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of recounting because they illustrate the possibilities of accomplishment when a goal is set and persistently sought, in season and out, regardless of obstacles or discouragements. The subject of our sketch early showed two outstanding characteristics—he was a nonconformist to many rules and customs and he had a burning desire to get into botanical work. The first got him into some tight places in college. There were two college regulations with which he came into conflict—the one was that all students had to do manual labor on the college farm and the other was that all had to participate in military drill. He tried to circumvent both and succeeded fairly well. Assisting one of the professors in the laboratory got him out of the farm work and taking some broadsword exercises sufficed for the military drill. The combination of his disregard for regulations and his love for botany operated together to get him into a difficulty that nearly prevented his graduation. In 1872 the American Association for the Advancement of Science met at Dubuque, Iowa. The great botanist, Asa Gray, was to be a speaker. Of course, a budding botanical student desired to be there. In fact, he just had to go. But the college authorities did not think it wise and would not give their approval to his petition to be absent long enough to attend the meeting. No amount of persuasion changed their verdict. What did our independent, ambitious student do? He went. And he graduated a little later, but many amends had to be made.

His desire to start on a career in botany, however, was not so easily satisfied. His first disappointment was that botany was not being taught when he arrived at the Iowa State College. Before going to college he had found a copy of *The American Agriculturist* in which the parts of a flower were illustrated and described. He collected the plants of his home region, analyzed the flowers, and sought names for them. Although no one taught botany when he arrived as a freshman in college, the professor of agriculture helped him by supplying the first botanical book he had ever seen. It was Eton's Manual of about 1840. But the first real help came during his sophomore year when Dr. Charles E. Bessey arrived at Ames as a member of the instructional staff in 1870. An immediate friendship developed between instructor and student. Professor Bessey must have been delighted to have such an enthusiastic student of botany and the student was certainly fortunate to have contact with such a gifted and inspiring teacher. In later years Professor Bessey referred to Dr. Arthur as his first "botanical son" and was justly proud of him.

The college course then made a fine start toward specialization in botany, thanks to Professor Bessey. There were courses in vegetable physiology and economic plants, and lectures on weeds and parasitic fungi. Dr. Bessey's description of the last-named course was that the subject was illustrated by means of a good microscope, the College Herbarium, and a large collection of fungous plants. Little wonder that a student of the proclivity of Joseph Charles Arthur laid there the foundation for a long career in mycology. But, once through college, the young graduate was severely tested. The modern era of botanical teaching and research was in its infancy. In 1872 botanical positions had not come into being in the colleges and universities. Agricultural experiment stations had not been founded, and there were no state or national departments of agriculture. Not until 1879, when he went to the University of Wisconsin as instructor in botany, did young Arthur succeed in obtaining a botanical position. But in the intervening years he never for a moment gave up the determination to make botany his life work, although he had to give attention to other things. He taught country schools several winters. He returned to the college at Ames several times to carry on special work. He prepared an exhibit for the Philadelphia Centennial Exposition in 1876. This consisted of a display of the flora of Iowa. For this he was awarded two diplomas and a bronze medal. In Philadelphia he worked for a time for a mineralogist. Then, going back to Iowa State, he became an assistant—not in botany but in zoology. He served also as an assistant in the library, where he helped to inaugurate the Dewey system of classification. His graduate work was in botany, the thesis being on the anatomy of *Echinocystis lobata* (wild cucumber-vine), and resulted in his receiving in 1877 the first Master of Science degree conferred by the College. For a while after that he had no work, but his yearning for an opportunity to work with plants led him to correspond with Peter Henderson, famous florist and seedsman of New York. This resulted in an opportunity for him to work in the Henderson greenhouses. This was in 1879. But the plan was never carried out. On his way to New York he stopped off at Baltimore to explore the city and more particularly the newly established Johns Hopkins University. A more or less chance interview with the President resulted in the offer of a complimentary fellowship of the value of $100.00. The opportunity to stay for a term was particularly welcome because the renowned mycologist, Dr. William G. Farlow, of Harvard, was coming within a week to begin a course of lectures. The Peter Henderson plan was abandoned. This spring term at Johns Hopkins was followed immediately with a summer term at Harvard. Work with Dr. Farlow was continued and other work was taken with Dr. George Lincoln Goodale and Dr. Asa Gray. What more could a struggling botanical student have desired? But there was more—John M. Coulter and Charles R. Barnes were fellow students. Lasting associations dated from that summer. Two or three years later Arthur, Barnes, and Coulter were working on the Handbook of Plant Dissection, which naturally came to be known as the ABC book. Botanical science is also indebted to these same three men for the *Botanical Gazette,* which they jointly edited over a long period of years.

In 1884 Joseph Charles Arthur was elected botanist in the newly founded Agricultural Experiment Station at Geneva. Here was a position for which he was well prepared and to which he was well suited. He had not been entirely happy in the teaching positions he had held just before at Wisconsin and Minnesota. The opportunity to do full-time research was exactly what he had desired. He made good from the start. His early work at Geneva with pear blight is known to all plant pathologists. While there he took advantage of the opportunity to do graduate work at Cornell, which brought him a doctor's degree in 1886.

In 1887 Dr. Arthur was called to Purdue University as Professor of Botany. The next year his title was changed to Professor of Vegetable Physiology and Pathology and Botanist to the Indiana Agricultural Experiment Station. This position he held until his formal retirement in 1915. From then until his death he was Emeritus Professor of Botany at Purdue. He retained his residence in Lafayette and continued his work at the University for many years. He was married to Emily Stiles Potter, of Lafayette, Indiana, in 1901, also a person of New England extraction. Mrs. Arthur died in 1935.

During the earlier years of his connection at Purdue Dr. Arthur taught plant physiology and plant pathology. He was much interested in designing and building apparatus for laboratory work in plant physiology, but he spent most of his time on research in plant pathology and mycology. His work on the cereal smuts and on potato scab was important scientifically and valuable economically. He was the first to use formaldehyde for the prevention of potato scab.

Although an important and pioneer contributor to other botanical fields, particularly physiology and pathology, Dr. Arthur must be most praised for his work with that intriguing group of fungi known as the plant rusts. For many years he has been recognized as a world authority on this group of fungi. His interest in the rusts began while he was about the college at Ames in the years following his graduation. Dr. Bessey had purchased a collection from the Curtis Herbarium. In it were many specimens named by M. J. Berkeley but there were also specimens which were unnamed. To the young investigator it seemed that names must be supplied. Then began the describing of new species in his first rust paper on "Iowa Uromyces" published in 1882. From then until the publication of his last paper in 1936 he was continuously investigating the rusts, their life-cycles, genetic relationships, and geographical distribution. In 1899 he began a series of culture studies which continued for nineteen years. The life-histories and host relationships of many species, possibly a hundred or more, were revealed by these painstaking experiments. In summarizing this work (see Memoranda and Index of Cultures of Uredineae, 1899–1917, Mycologia **13**: 230–262, 1921) Dr. Arthur states that more than 2390 collections were used and that about 3750 sowings were made. When it is realized that almost every culture involved a potted plant growing in a greenhouse some idea of the magnitude of the work may be gained. Almost more phenomenal is the fact that more than 85 botanical correspondents contributed specimens and field observations year after year. A large number of these correspondents were not professional botanists and many of them acquired their interest, ability, and inclination either through personal contacts or correspondence with

Dr. Arthur. It is a marvelous example of how invaluable voluntary assistance may be enlisted through boundless enthusiasm and fine inspiration. Throughout his long professional career Dr. Arthur's work was never limited to the facilities and resources of the institution with which he was connected but extended far beyond that range.

In 1905 he brought out a new classification for the order Uredinales. Although he modified this classification in later years it served in its time as a great impetus to the researches on the group. In 1907 he began the preparation of a taxonomic treatment of the North American Uredinales for the *North American Flora* (published by the New York Botanical Garden). This ran into 11 parts consisting of a total of 765 pages and required 20 years for completion. Two books have been published—a volume titled *The Plant Rusts* in 1929 (in collaboration with F. D. Kern, C. R. Orton, F. D. Fromme, H. S. Jackson, E. B. Mains, and G. B. Bisby, all former associates in his laboratories), a biological treatment, and a *Manual of the Rusts in United States and Canada*, in 1934, a taxonomic treatment. In addition to these larger publications he found time to bring out a very long list of papers dealing with varied aspects of the rusts. Many collectors sent him their specimens. He determined and reported on collections not only from the United States but from Canada, Cuba, Puerto Rico, Mexico, Guatemala, South America, and the Philippines. He made collecting trips himself to the Rocky Mountains, New England, several southeastern States, and to Texas, New Mexico, and Arizona in the Southwest. He made numerous trips to Europe partly because of his fondness for travel but mostly to study type specimens in some old world Herbarium or Botanical Garden, to obtain access to some rare literature, or to confer with fellow workers, or to attend an International Botanical Congress where botanical nomenclature was under discussion. He was naturally much concerned with the rules of nomenclature, not only as they applied to his group of rusts, but also as they applied to the host plants. He was active in the Congresses at Vienna, 1905, Brussels, 1910, and Cambridge, 1930. In 1925 a trip was planned especially to confer with European mycologists who had special interests in the rusts. It was the privilege of the writer to accompany Dr. Arthur on this trip. Visits were made with Klebahn, Sydow, Dietel, Kniep, Lagerheim, Eriksson, Juel, Jørstad, Gäumann, Ramsbottom, Butler, and Miss Wakefield. A paper in *Science* (Vol. 43, pp. 558–560), entitled Conversations with European Mycologists, illustrates the value of exchanging opinions with fellow botanists in Germany, Sweden, Norway, Switzerland, and England. It was a strong link in a chain of cosmopolitan activities forged by Dr. Arthur throughout his long life.

It is difficult to render an adequate appraisal of Dr. Arthur's contributions to our knowledge of the plant rusts. They are set forth in 150 papers over a period of more than fifty years. The total number of papers on all subjects is 289. No detail was ever too small to be considered and no effort was too great if there was a possibility of arriving at a clearer understanding of the identity and relationships of the various forms and species. Nor can we measure the results merely by reviewing his own publications but must take into consideration how he reached out into the lives of his students, his assistants, his correspondents, and his colleagues, spurring them on to an ever widening circle of activities. Some of his associates, who in their own turn have been active, have already been named as collaborators in the book on The Plant Rusts. We would fail if we did not name Bolley, Olive, Whetzel, Johnson (A. G.), Christman, Dodge, Garrett, Bethel, Holway, Rosen, Stevens (F. L.), Clinton, Kunkel, Thurston, and Cummins, who were influenced either directly or indirectly. There are many others whose contributions were less extensive but nevertheless important. It is not given to many men to be so productive both through their own efforts and through their influence on the efforts of others.

Dr. Arthur was a member of Sigma Xi; the American Society of Naturalists; the American Philosophical Society; the Academy of Natural Sciences of Philadelphia; the American Academy of Arts and Sciences; the Indiana Academy of Science (president, '93); the American Association for the Advancement of Science (vice president, '95); the Society for the Promotion of Agricultural Science; the Botanical Society of America (twice president, '02, '19); the Torrey Botanical Club; the American Phytopathological Society (president, '33); the Mycological Society of America; the Deutsche Botanische Gesellschaft; and the Russian Botanical Society.

The life and work of Dr. Arthur should serve as a great inspiration to ambitious young workers. A pioneer spirit, real resistance to discouragement, industrious habits, sound scholarship, unflagging persistence, and singleness of purpose led Dr. Arthur to high achievement.

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A TOXIMETRIC STUDY OF SOME ERADICANT FUNGICIDES[1]

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INTRODUCTION

Chemical eradication is an accepted practice in the control of several major plant diseases and offers possibilities for wider use. In recent years renewed interest in this means of plant disease control has been stimulated by an increasing knowledge of epidemiology and by frequent failures of control programs that relied solely on applications of protectant fungicides. Special attention has been given to the feasibility of eradicant spraying as an aid in the control of certain diseases of orchard and small-fruit crops, particularly apple scab, caused by *Venturia inaequalis* (Cke.) Wint. (12, 16, 20). Several investigators (1, 2, 3, 7, 8, 10, 11, 13, 14, 15, 16, 17, 20, 21, 22, 23, and 28) have reported a drastic reduction of the ascosporic inoculum of this fungus by experimental applications of eradicant fungicides to the overwintered leaves on the orchard floor. Of the materials that have been thus tested, a proprietary preparation of sodium dinitro-ortho-cresylate, trademarked Elgetol, seems to have given the most satisfactory results.

The first comprehensive work on the toxicity to fungi of sodium dinitro-ortho-cresylate was reported in 1912 by Falck (5). Among the many cresol and phenol derivatives tested, the sodium and potassium salts of dinitro-ortho-cresol were the most toxic. Truffaut and Pastac (29), 1932, recommended the use of Elgetol, developed by them during a study of fungicidal organic dyes, as a fungicide and insecticide for dormant spraying. They cited numerous phytopathogens and insect pests against which it might be used. Pastac (25) has given further data on the toxicity of some dinitro compounds.

The purpose of the *in vitro* experiments reported here has been to study comparatively the toxicity of Elgetol and some other potential eradicant fungicides.

The test fungi were *Venturia inaequalis* and some other plant pathogens that might be combated by eradicant spraying.

The test materials, in addition to Elgetol, were Lignasan (a commercial preparation of ethyl mercury phosphate) and preparations of phenyl mercury oleate and a toluene derivative, respectively. Limited data of Keitt, Clayton, and Langford (16) have indicated that Lignasan has high toxicity to the scab fungus in overwintered leaves. The other 2 materials were chosen to include another organic mercurial compound and a toluene derivative that showed a promising degree of toxicity in preliminary tests.

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The procedure was based on the agar-plate method developed for toximetric studies of wood preservatives by Schmitz and others (27). Palmiter and Keitt (24) adapted the method to an *in vitro* study of eradicant fungicides. Humphrey and Fleming (9) have reviewed the methods and materials used in the early toximetric investigations of wood preservatives.

The method is adapted to a preliminary evaluation of the toxicity of potential fungicides and studies on their toxic action under the experimental conditions. Tests may be made without seasonal limitations, rapidly, and in a controlled environment. Critiques of the method, as applied to the testing of wood preservatives, have been presented by Bateman (4), Findlay (6), and Richards (26).

This work is designed to supplement field studies and aid in the interpretation of their results. It is recognized that the findings from such work cannot be applied directly to field practice because of the differences in conditions.

In addition to the *in vitro* studies, limited experiments were made on the suppression of the ascosporic inoculum of *Venturia inaequalis* in naturally overwintered apple leaves.

TOXIMETRIC STUDIES

Methods

The fungicides were pipetted aseptically from freshly prepared self-sterilized stock solutions or suspensions into proper volumes of cool sterile distilled water in 250-cc. Erlenmeyer flasks to give 100 cc. of fungicide at twice the desired final concentration. As certain of the fungicides had volatile components, none of them was heated at any time. The medium (Trommer's extract of malt, 25 g.; agar, 17 g.; and distilled water to make 1000 cc.) was freshly prepared at twice the final concentration, dispensed without filtering in 100-cc. lots into 250-cc. flasks, and autoclaved at 15 pounds' pressure for about 25 minutes. The medium was never reheated after the first sterilization.

The diluted fungicide was added to the partially cooled medium, shaken well, and about 50 cc. of the mixture poured into each of four 12-ounce bottles (Illinois-Owens ovals) with metal screw caps containing oiled-paper inserts. The bottles were laid on their broad plane surface while the medium gelled. For toxic materials in suspension, the temperature was regulated to provide rapid gelling. In adapting the method for the present study, Petri dishes were replaced by bottles to obviate appreciable losses of water and volatile components of the toxic agents.

The plates from which disks were cut for planting the bottles contained 15 to 17 cc. of malt-agar medium and were planted with a standardized water suspension of mycelium and spores. They were wrapped with wax paper and incubated in darkness at 70° F. for the following periods: *Sclerotinia fructicola* (Wint.) Rehm, 5 days; *Coryneum beijerinckii* Oud. and *Valsa cincta* Fr., 7 or 8 days; *Cladosporium carpophilum* Thüm, and

Coccomyces hiemalis Higgins, 14 or 15 days; and *Venturia inaequalis*, 16 or 17 days. Disks of *Valsa cincta* were cut before the pycnidia were visible or when only immature pycnidia were present.

Disks 5 mm. in diameter and of uniform thickness were cut from vigorous mycelia of the test fungi. Six disks with the fungus-bearing surface uppermost were placed about equidistant from each other on the medium in the bottle. The bottles were inverted, incubated in a horizontal position, and kept in darkness at 70° F. for 2 weeks. The liquid that accumulated on the lower inside surface of the bottle during the incubation period was carefully poured out before the disks were removed.

After the incubation period, the disks without macroscopic evidence of mycelial growth, subsequent to planting, were removed to Petri dishes containing about 20 cc. of malt agar and placed with the fungus-bearing surface next to the medium. The plates were inverted and incubated for 3 weeks. The fungus was considered dead if new growth did not appear. Each bottle was considered as a unit, being listed as positive if growth occurred on any disk. Care was taken to handle each disk separately when testing for viability, and all were recorded individually in the original data.

The criteria of toxicity are comparable to those used by Palmiter and Keitt (24): *growth* represents the highest concentration at which survival occurred in any series of tests, and *death* represents the lowest concentration at which killing always occurred in any series of tests.

Duplicate bottles for each concentration of fungicide and each isolate under consideration were prepared in every test. Every test to determine the critical data was done at least 3 times with freshly prepared fungicide and nutrient medium. Duplicate bottles containing plain malt-extract agar were planted for each isolate concerned in every experiment. The fungus always grew well in these bottles.

The test fungi grew well throughout the range of pH encountered in this work. Plain malt-extract agar had a reaction of pH 5.5 to pH 6.0. Every fungicide reduced the acidity of the medium, usually to the range of pH 6.0 to pH 6.5. At the higher concentrations of Elgetol the reaction of the medium was about pH 7.0. There were no appreciable pH changes during the test period, except when the fungus grew on the toxic substratum. Then the reaction shifted towards pH 5.5, the magnitude of the shift depending upon the extensiveness of the mycelial growth. All pH measurements were made with a Coleman Model–3 pH Electrometer.

It is recognized that minor errors in percentage concentration of the toxic substratum are inevitable in dealing with the methods and materials used here. All feasible precautions were taken to minimize such errors.

Materials

Description and Source of the Toxic Preparations

Elgetol: The Elgetol used was a liquid proprietary preparation containing 19.4 per cent by weight of sodium-dinitro-ortho-cresylate with water and a penetrant added.

Lignasan: The Lignasan used was a powdered proprietary fungicide containing 6.25 per cent by weight of ethyl mercury phosphate mixed with non-toxic inert material.

FD–2: Phenyl mercury oleate was used in a preparation coded FD–2 that contained 10 per cent by weight of the toxic agent. The remainder of the product was mineral oil and an emulsifier.

IN–3102 A8: A toluene derivative was used in a preparation coded IN–3102 A8 that contained 50 per cent by weight of the toxic agent.

At the concentrations used, Elgetol was completely in solution, Lignasan and the toluene derivative preparation were primarily suspensions, and the phenyl mercury oleate preparation was dispersed in an emulsion.

The data on the composition of each material listed above are based on the manufacturer's statement. Elgetol was furnished through the courtesy of Standard Agricultural Chemicals, Incorporated. Lignasan, the phenyl mercury oleate preparation, and the toluene derivative preparation, were provided through the courtesy of E. I. du Pont de Nemours and Company.

Source and Purity of the Test Fungi

The test fungi were obtained from the following sources: *Cladosporium carpophilum,* a monoconidial isolate from a peach twig furnished by Donald Cation, East Lansing, Michigan, 1939; *Coccomyces hiemalis,* 2 monoconidial isolates from Montmorency cherry leaves at Madison, Wisconsin, 1940; *Coryneum beijerinckii,* a monoconidial isolate from each of 2 cultures isolated from peach and furnished by E. E. Wilson, Davis, California, 1939; *Sclerotinia fructicola,* a monoconidial isolate from each of 2 plums collected at Gays Mills, Wisconsin, 1939; *Valsa cincta,* a monoconidial and a monoascosporic isolate from E. M. Hildebrand, Ithaca, New York, 1939; *Venturia inaequalis,* 2 monoascosporic isolates of opposite sexual compatibility groups from the same ascus, obtained by M. H. Langford at Madison, Wisconsin, 1937 (*cf.* 19). Isolate B4 is here designated as isolate *1,* and B8 as isolate *2.*

All of the isolates except those of *Valsa cincta* were propagated by monoconidial transfers, and all remained true to type, as judged by macroscopic characters. Usually 2 isolates of each organism were used to guard against the possibility of limiting the work to a single isolate of unusual reaction.

EXPERIMENTAL RESULTS AND DISCUSSION

Toxicity of the Fungicides to the Test Fungi

The data from the individual tests, based on the criteria of toxicity defined elsewhere, are summarized in table 1. Some of the critical data indicate the maximum tolerance of the isolate as expressed in 1 divergent test. This occurred especially with the non-mercurial preparations. As the lethal concentration of a fungicide was approached, the number of disks surviving usually diminished, until often the critical concentrations were determined by the survival of only 1 or 2 of the 12 disks in duplicate bottles.

Coccomyces hiemalis was constantly one of the more susceptible species, usually followed in order by either *Venturia inaequalis* or *Valsa cincta.* *Cladosporium carpophilum*, *Coryneum beijerinckii*, and *Sclerotinia fructicola* were commonly the more resistant organisms.

Differential susceptibility to the toxic action of a given fungicide occurred among the species of test fungi. This was accentuated by the weaker toxic preparations. The reaction of each isolate was quite uniform in a series of tests, considering the relative toxicity of the fungicide used, and noteworthy differences between isolates of the same organism were not found. No correlation between the cultural characteristics of the several isolates and their susceptibility to toxic action was evident.

TABLE 1.—*Toxicity of 4 potential eradicant fungicides to 6 phytopathogenic fungi*

Test fungus and isolate number		Criteria	Critical concentrations[a] for			
			Elgetol	Lignasan	Phenyl mercury oleate preparation	Toluene derivative preparation
			Per cent	*Per cent*	*Per cent*	*Per cent*
Cladosporium carpophilum	1	Growth	0.075	0.0025	0.010	....
		Death	0.100	0.0050	0.025	> 0.200
Coccomyces hiemalis	1	Growth	0.010	0.0010	0.005	0.025
		Death	0.025	0.0025	0.010	0.050
	2	Growth	0.010	0.0010	0.020	0.010
		Death	0.025	0.0025	0.030	0.025
Coryneum beijerinckii	1	Growth	0.050	0.0025	0.040	0.100
		Death	0.075	0.0050	0.050	0.200
	2	Growth	0.100	0.0010	0.040	....
		Death	0.200	0.0025	0.050	> 0.200
Sclerotinia fructicola	1	Growth	....	0.0025	0.020	0.020
		Death	> 0.200	0.0050	0.030	0.030
	2	Growth	0.100	0.0050	0.030	0.030
		Death	0.200	0.0075	0.040	0.040
Valsa cincta	1	Growth	0.025	0.0025	0.005	....
		Death	0.050	0.0050	0.010	> 0.200
	2	Growth	0.050	0.0010	0.020	....
		Death	0.075	0.0025	0.030	> 0.200
Venturia inaequalis	1	Growth	0.050	0.0010	0.005	....
		Death	0.075	0.0025	0.010	> 0.075
	2	Growth	0.050	0.0010	0.005	....
		Death	0.075	0.0025	0.010	> 0.075

[a] The figure shown for growth is the highest concentration at which survival occurred in any series of tests; for death, the lowest that always killed.

> indicates that the lethal concentration was greater than the figure shown. Percentage concentrations are based on the toxic preparation as described in the text.

Under the experimental conditions, Lignasan was the most toxic fungicide, followed by the phenyl mercury oleate preparation, Elgetol, and the toluene derivative preparation. Based on equal percentage concentrations of the toxic agent, the toxicity of Lignasan was 3 to 300 times that of any other preparation, depending upon the fungicide and the isolate used. The phenyl mercury oleate preparation on this basis was considerably more toxic than Elgetol.

In considering the relative toxicity of these fungicides, allowance should be made for the advantages accruing to preparations yielding a toxic vapor. Except Elgetol, every preparation had a vapor that was toxic at the concentrations used, and that of Lignasan was especially toxic, as proved by limited experiments not reported in detail.

The specificity of the fungicides was brought out by the reactions of the several test fungi. Each mercurial preparation was consistently toxic within its own range to all of the isolates. Some evidence of specificity was found with Elgetol. The toluene derivative preparation was markedly toxic to only 2 test fungi, *Coccomyces hiemalis* and *Sclerotinia fructicola*, ordinarily a resistant species in this study and in that of Palmiter and Keitt (24).

Relation of Concentration of Toxic Preparation to Time Required for Killing Fungus

Indications were sought as to the time required for a given concentration of a fungicide to kill a particular fungus under the conditions used for studying relative toxicity. Elgetol and Lignasan were the fungicides used; the fungi were 2 isolates of each *Coryneum beijerinckii, Sclerotinia fructicola*, and *Venturia inaequalis.*

The methods of preparing the toxic substratum and the plates from which disks were cut were the same as those described earlier. Each bottle was planted with 6 disks and incubated for a suitable period under conditions given above. Thereafter, the disks of each bottle were removed to a Petri dish containing about 30 cc. of sterile distilled water, leached for approximately 1 hour, and then placed with the fungus-bearing surface down on malt-agar plates, which were inverted and incubated at 70° F. for 3 weeks. Disks that did not show signs of life after this interval were considered dead. Each bottle was considered as a unit, for the disks mingled during leaching.

The leaching extended the time during which the fungus was in contact with the fungicide, but this source of error is regarded as unimportant. The vitality of the fungus was not affected perceptibly by immersion, as shown by tests of untreated fungus-bearing disks.

The critical data were obtained by using each fungus with each concentration of the 2 fungicides and at each critical time interval in at least 3 tests, with 2 exceptions. Only 2 tests were made with *Sclerotinia fructicola* and Elgetol, at 0.25 per cent, after earlier data showed that Elgetol, at 0.10 per cent, was not lethal to either isolate in the 14-day test. One test was made with this fungus and Lignasan at 0.005 per cent. The results of the several tests in this part of the study were reasonably uniform.

Figures 1 and 2 indicate the maximum survival period for each isolate in any test of a given concentration of fungicide. Killing occurred at some time during the last recorded interval on the bar graph.

With both Elgetol and Lignasan, *Sclerotinia fructicola* was the most

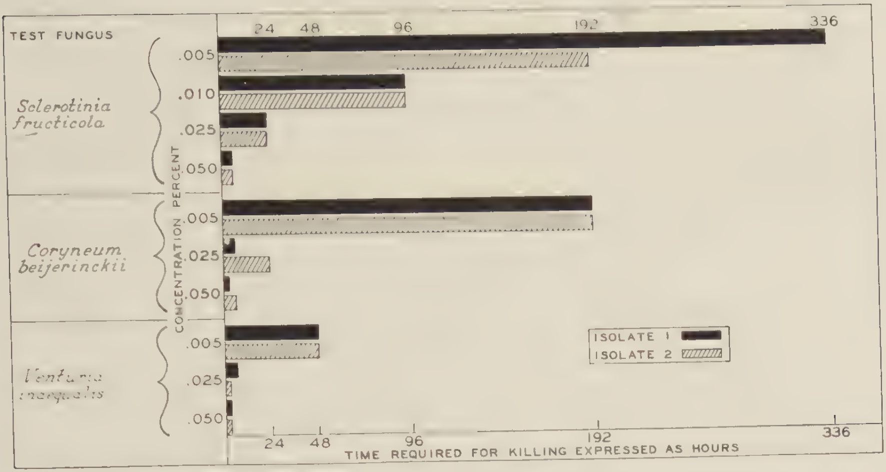

FIG. 1. Relation of the concentration of Lignasan to the time required for killing certain fungi.

resistant species, followed in order by *Coryneum beijerinckii* and *Venturia inaequalis*. Lignasan was the more toxic of the fungicides. The difference in susceptibility noticeable among the test fungi at the lowest concentration of Lignasan may be misleading unless it is observed, in table 1, that the concentration of 0.005 per cent was about a minimum lethal dose for *Sclerotinia fructicola*, 1 to 2 times that for *Coryneum beijerinckii*, and 2 to 5 times that for *Venturia inaequalis*. At a concentration of 0.025 per cent, the relations to the minimal lethal dose for these fungi were, respectively, 5, 5 to 10, and 10 to 25; at 0.05 per cent, 10, 10 to 20, and 20 to 50.

The fungi were killed rapidly at the higher concentrations of Lignasan. At 0.025 per cent, all of the isolates were killed within 24 hours. No more than 6 hours was required for killing at 0.05 per cent. Lignasan probably had important advantages over Elgetol in these tests because of its highly

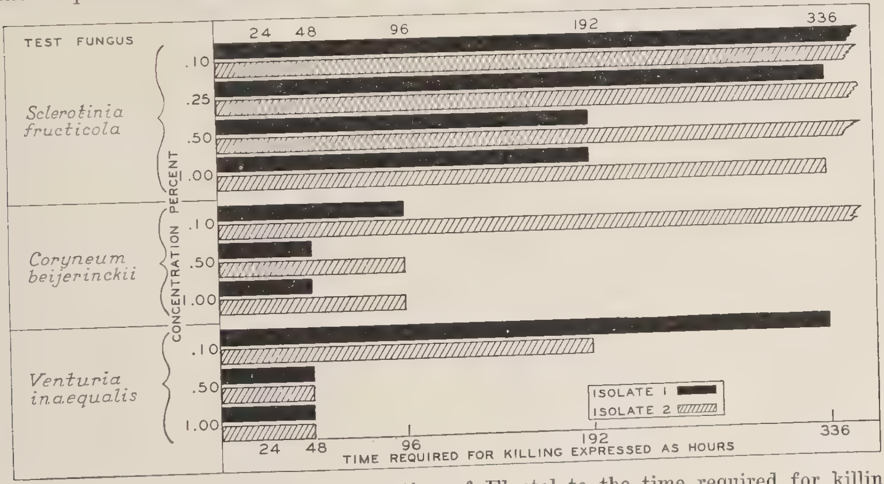

FIG. 2. Relation of the concentration of Elgetol to the time required for killing certain fungi.

toxic vapors and the use of an airtight testing chamber. Even at the highest concentration of Elgetol, *Sclerotinia fructicola* was markedly resistant, whereas the most resistant isolates of the other test fungi were killed within 48 to 96 hours.

Elgetol, at 0.5 per cent, was about twice the minimum lethal dose for *Sclerotinia fructicola,* 2 to 5 times that for *Coryneum beijerinckii* and 7 times that for *Venturia inaequalis.* At the concentration of 1.0 per cent, the relations were, respectively, about 5, 5 to 10, and 13.

Suppression of Ascosporic Inoculum of *Venturia inaequalis*

Data with which to supplement the *in vitro* experiments were sought in experiments at the field laboratory at Gays Mills, Wisconsin. Overwintered apple leaves bearing numerous perithecia of *Venturia inaequalis* were selected under trees of the Cortland variety on April 9, 20, and 25, respectively, for the 3 experiments reported here. Only leaves overwintered on sod with their dorsal side uppermost were used. Suitable numbers of leaves were arranged out-of-doors on sod under wire netting with the weathered surface exposed and no overlapping. They were sprayed with Elgetol, at 0.5 per cent, at the rate of 600 gallons per acre. A knapsack type Meyer's sprayer was used.

After the leaves were dry—within 2 hours after spraying—a single disk 15 mm. in diameter was cut from each in an area where ascocarps were abundant. At intervals of 3, 6, 12, and 24 hours after the leaves were sprayed, 20 disks for each time interval were leached in 1 liter of tap water for an hour. Then 5 disks were placed on the inside surface of the cover in each of 4 Petri dishes, the excess water was removed, and the perithecia were allowed to discharge ascospores for several hours on agar gel made aseptic by copper sulphate. Each disk was set up 4 consecutive times for ascospore discharge over the same area of the agar plate. Between discharges the disks were kept under conditions suitable for maturing of more ascospores. The discharges were made at 3- or 4-day intervals and at room temperature. Unsprayed checks of 20 disks each received the same treatment during the period of ascospore discharge. The abundant ascospore discharge from the perithecia in untreated leaf disks indicates that the experimental material and procedure were suitable for the purpose (Table 2).

The ascospores were counted under the microscope without duplication by manipulating the open Petri dish on a mechanical stage. When no more than a few hundred spores were present, all were counted or closely estimated. If several hundreds or thousands of spores were present, 30 fields per individual area were counted or closely estimated and the total discharge calculated.

The indicated reduction in ascospore discharge from perithecia in leaf disks treated with Elgetol varied little in the 3 experiments, usually amounting to more than 99 per cent (Table 2).

Evidently, Elgetol acts quickly, for the suppression after 3 hours was as complete as that at the longer intervals. The results of a single experiment suggest that rapid drying may hasten the inactivation of the fungus. In experiment 3, parallel series of 20 disks each were prepared for the 3-, 6-, and 12-hour intervals. The disks of 1 series were not allowed to dry during the interval from spraying to leaching, and the other series was handled in the usual manner. In leaves allowed to dry rapidly, the suppression was almost 100 per cent by the end of the 3-hour interval. Six hours were required for equal suppression of the fungus in the constantly wet leaves, although the suppression was about 98 per cent after the 3-hour treatment.

TABLE 2.—*Time required for suppression of the ascosporic inoculum of Venturia inaequalis by Elgetol applied to overwintered leaves of the Cortland variety, Gays Mills, Wisconsin, 1941*

Period of treatment in hours	Total ascospore discharge from 20 disks[a]			Indicated percentage reduction in discharge by treatment		
	Experiment No.			Experiment No.		
	1	2[b]	3	1	2	3
Untreated check	424,676	743,213	752,911			
3	92	19,818	5	99.9	97.9	99.9
6	204	4,090	534	99.9	99.6	99.9
12	707	21,715	144	99.8	97.7	99.9
24	149	12,192	1,340	99.9	98.7	99.8

[a] A single disk 15 mm. in diameter was cut from an area of a leaf where ascocarps were abundant.

[b] Most of the spores were from 1 or 2 disks: 3 hours, 2 disks with 11,084 and 5,444 spores, respectively; 6 hours, 1 disk with 2,406 spores; 12 hours, 1 disk with 18,635 spores; and 24 hours, 1 disk with 10,413 spores.

Interest attaches to the question whether Elgetol treatment can effectively suppress the ascosporic inoculum of the scab fungus if applied after the ascospores are ripe and ready for discharge. The developmental stages of the ascocarps and the course of natural discharge of ascospores at the time of the experiments were studied. Both fresh and preserved ascocarps were examined in the study of developmental stages. The preserved material was chosen from treated leaves just before the disks were set up for the first ascospore discharge in each experiment and was fixed in formal-acetic alcohol, embedded in paraffin, sectioned, and stained in 0.05 per cent aqueous cotton blue. Perithecia were also picked from the preserved leaves, crushed, and examined.

Ascospores were starting to mature at the beginning of the first experiment, so that a few olivaceous spores were present in some of the perithecia. At the beginning of the second and third experiments, many of the ascocarps contained not less than 5 to 10 asci with olivaceous spores (Fig. 3).

Evidence on natural discharge of ascospores was obtained by the method of Keitt and Jones (18). The first natural discharge was observed on April

9, and small to moderate discharges occurred by the time of the second and third experiments.

It is noteworthy that the suppression of the ascosporic inoculum in the second and third experiments was as effective as in the first.

The *in vitro* experiments reported elsewhere in this paper necessitated the use of killing as the criterion of effectiveness of the several fungicides. In practice, the goal of chemical eradication is the suppression of the inoculum of a phytopathogen. Consequently, immediate killing may be of secondary importance. It is significant that Elgetol, at 0.5 per cent, drastically suppressed the inoculum of *Venturia inaequalis* in overwintered apple leaves within 3 hours, whereas, in *in vitro* experiments, 24 to 48 hours elapsed before all of the fungus was killed at this concentration.

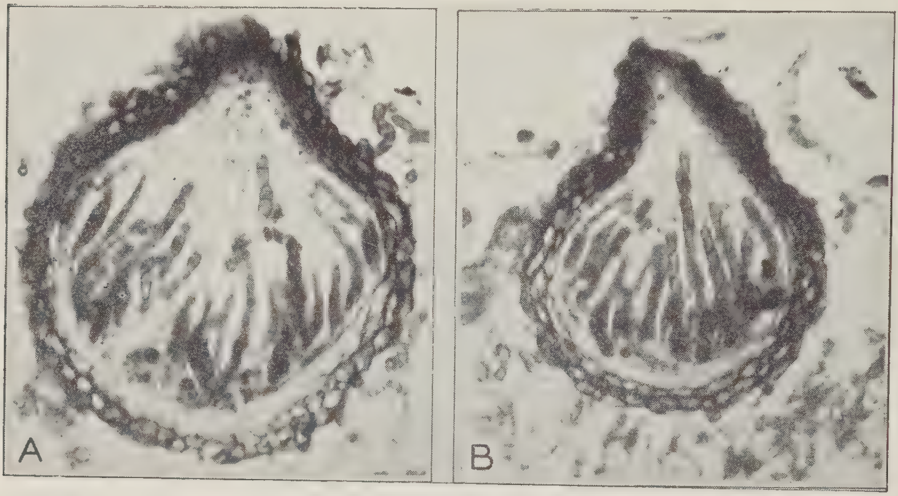

FIG. 3. Sections of perithecia of *Venturia inaequalis* from material fixed at the beginning of the second experiment, illustrating the more advanced developmental stages observed. A. Untreated perithecium. B. Perithecium treated with 0.5 per cent Elgetol. Both approximately × 330.

A satisfactory suppression of the inoculum of certain fungi is most sure with toxic preparations that act quickly. For instance, the apple-scab pathogen and *Coccomyces hiemalis*, the cherry leaf-spot pathogen, develop on the orchard floor in overwintered leaves from which spray residue may be leached by rain. A preparation that at feasible concentrations would render a sufficient part of the potential inoculum harmless within a few hours is the type of material sought. There are strong indications from the orchard trials of other investigators that Elgetol and Lignasan approach this requirement. The results of the field experiments reported herein give additional evidence that Elgetol is capable of rapid and drastic suppression of the ascosporic inoculum of *Venturia inaequalis*.

SUMMARY

The toxicity of 4 potential eradicant fungicides was studied *in vitro* by a modification of the agar-plate method of Schmitz and others, 6 species of phytopathogens being used as test fungi. Limited studies were made of the suppression of the ascosporic inoculum of *Venturia inaequalis* in overwintered apple leaves by Elgetol.

Coccomyces hiemalis was constantly one of the more susceptible test fungi, followed in order by either *Venturia inaequalis* or *Valsa cincta. Cladosporium corpophilum, Coryneum beijerinckii*, and *Sclerotinia fructicola* were more resistant. The relative susceptibility of the organisms varied with each toxic preparation. Each isolate reacted quite uniformly in a series of tests, and the differences between isolates of the same species were not noteworthy.

Under the experimental conditions, Lignasan was the most toxic fungicide, followed in order by the phenyl mercury oleate preparation, Elgetol, and the toluene derivative preparation. These materials usually killed at relatively low concentrations. Each, except Elgetol, had a toxic vapor at the concentration used, an advantage that should be considered in interpreting relative toxicities. Specificity of toxic action was shown clearly only by the toluene derivative preparation.

Venturia inaequalis was killed within 3 hours by Lignasan and within 24 to 48 hours by Elgetol at the highest concentrations used in the *in vitro* experiments. *Coryneum beijerinckii* was more resistant, and *Sclerotinia fructicola* was the most resistant organism to both fungicides. Although Elgetol killed more slowly than Lignasan, the apparent difference in rate of killing becomes less important if the relation between the minimum lethal dose and the concentration used for each fungus and fungicide is considered.

Elgetol at 0.5 per cent, used at the rate of 600 gallons per acre, usually suppressed more than 99 per cent of the ascosporic inoculum of *Venturia inaequalis* in naturally overwintered apple leaves. Suppression was as complete 3 hours after treatment as later. It was possible in these experiments to limit drastically the ascospore discharge from perithecia containing ripe spores.

The importance of the time required for adequate suppression of the ascosporic inoculum of certain fungi is discussed.

The results reported herein give additional evidence that Elgetol is capable of rapid and drastic suppression of the ascosporic inoculum of *Venturia inaequalis*.

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THE EFFECT OF MOSAIC VIRUS INFECTION ON THE PROTEIN CONTENT OF SUSCEPTIBLE AND RESISTANT STRAINS OF TOBACCO[1]

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INTRODUCTION

It is known that the common-mosaic virus causes an alteration in the normal protein synthesis and that the multiplication of virus in susceptible strains of tobacco is influenced by the nitrogen nutrition of the plants.

Stanley (17) found that the extracts from the mosaic-diseased plants contained more total nitrogen and more protein than the extracts from healthy plants of the same age. Bawden and Pirie (4) also observed the abnormally high protein content of the expressed juice from mosaic-diseased tobacco plants.

Spencer (15) found that Turkish tobacco plants grown on a very low nitrogen level produced little, if any, virus, whereas an increased nitrogen supply accelerated the synthesis of virus protein.

The changes in various protein fractions of mosaic-diseased tobacco were studied by Martin, Balls, and McKinney (10, 11). However, since all the protein of the plant is not in the expressed juice, they made use of the tissues, as well as the juice, in their determinations. They concluded that during the early stages of infection the virus protein accumulates by displacing an equal amount of normal protein. At a later stage the virus protein is present in addition to the normal amount of soluble proteins maintained by healthy plants. They made the interesting observation that some strains of tobacco that are extremely resistant to the mosaic virus show a decrease in total nitrogen content as a result of virus infection (11). This response is entirely opposite to that observed in the susceptible varieties of tobacco investigated.

In the present study we have investigated further the differences in the protein metabolism and the oxidizing enzyme contents of susceptible and resistant types of tobacco. Such studies are of value in arriving at an understanding of the mode of virus synthesis in plants and the basis of virus resistance exhibited by various genotypes of tobacco.

EXPERIMENTAL

Materials and Methods

The tobacco plants used in this study were of two types: (1) *Nicotiana tabacum* L., var. Wisconsin-Havana Seed—a mosaic-susceptible variety—

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[1] Studies conducted under Bankhead-Jones Project S.R.F. 2–17, U. S. Department of Agriculture, Bureau of Plant Industry and Bureau of Agricultural Chemistry and Engineering cooperating.

[2] Associate Chemist, Bureau of Agricultural Chemistry and Engineering; senior pathologist, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C., respectively.

and (2) T.I. No. 448A, a genotype of common tobacco, which was found by McKinney (8) to possess the highest degree of resistance to the tobacco-mosaic virus known in tobacco. Although T.I. No. 448A develops a low concentration of virus in the leaves, it does not develop the usual mosaic symptoms, and the virus is not completely systemic in the plant.

The plants were grown in the greenhouse in pots during the winter and spring. Temperatures were controlled near a mean of 22.5° C., but this temperature was exceeded on days when the sun was bright. Plants were inoculated with *Nicotiana Virus 1* when the largest leaves were from 3.5 to 5 cm. wide. In the low-nitrogen series the plants were potted with a very poor soil, low in humus and soluble nitrogen. The series designated as "good soil" was grown in composted greenhouse soil made up from fertile field soil and composted manure. In the high-nitrogen series, the plants were potted in "good soil" and to each pot was added one pint of a 1.0 per cent solution of calcium nitrate twice a week.

Plant collections were made when the symptoms became fully developed in Wisconsin-Havana Seed tobacco or, in some instances, at an arbitrary time after inoculation. Four or more plants from each set were cut at the point of inoculation and all the leaves above the inoculated leaf, including the tip leaves, were removed and the stems discarded. The leaves were then cut into small pieces with a knife and mixed to give uniform samples for the determination of dry matter and total nitrogen. Total nitrogen was determined on the oven-dry samples by the usual Kjeldahl method, except that the sulphuric acid used for the Kjeldahl digestion contained 3 g. salicylic acid per 100 cc. of acid.

The remaining fine-cut leaf tissue was frozen and later thawed and extracted with an equal weight of M/10 phosphate buffer, pH 7.0. After standing at room temperature for 2 or 3 hours the tobacco-leaf extract was squeezed through cheesecloth and clarified by centrifuging. Aliquots of this extract were used for the determination of soluble-protein nitrogen, nonprotein nitrogen, virus nitrogen, and virus assays on bean leaves by the paired-leaf method.

The nonprotein nitrogen was measured by the iron-reduction method of Pucher, Leavenworth, and Vickery (13). Protein nitrogen was determined by precipitation with 2.5 per cent trichloroacetic acid and heating at 75° C. for 10 minutes. The precipitate was resuspended in a 10-cc. centrifuge tube with 2.5 per cent trichloroacetic acid and then centrifuged. The washed precipitate was dissolved by gently heating with 1 cc. of concentrated sulphuric acid and then transferred to a micro-Kjeldahl digestion flask. The nitrogen was determined by the micro-Kjeldahl method.

Virus-protein nitrogen was determined by the isoelectric precipitation method described by Hills and McKinney (7). In brief, the procedure was to acidify a 40-cc. aliquot of the leaf extract to pH 4.2–4.0, at which point a fraction of nonvirus protein precipitates and can be removed by centrifuging. On careful acidification to pH 3.4 the virus nucleoprotein is obtained in the precipitate and the virus-protein nitrogen can then be determined in the same manner as protein nitrogen.

Biological assays of the virus were made by the paired-leaf method on the primary leaves of 8- to 12-day-old bean seedlings (*Phaseolus vulgaris* L., var. Scotia).

The determinations of chlorophyll and chlorophyllase in fresh green leaf tissue were made by the method used by Peterson and McKinney (12). The midribs and petioles were removed in order to get more uniform and reliable leaf samples.

The extracts used for the estimation of oxidase, catalase, and peroxidase were prepared by grinding 10 g. of fresh, fine-chopped leaf tissue in a mortar with sand and 100 cc. of a mixture of 1 part glycerol and 1 part of M 5 phosphate buffer, pH 8.0. The suspension was centrifuged at a very slow speed (about 700 r.p.m.) for 5 minutes to remove coarse fiber and sand. The solution was stored in a refrigerator at 0° C. Determinations were made within 3 days after preparation of the glycerol extracts. It was found that the enzymes being studied did not deteriorate measurably in 3 days' storage at 0° C.

Oxidase activity was measured by Guthrie's (6) iodometric method. Catalase and peroxidase were determined by the methods published by Balls and Hale (2, 3), except that it was found convenient to remove aliquots immediately and after 4, 8, and 12 minutes instead of after the time intervals suggested by those authors. Catalase and peroxidase activities are expressed as the reaction velocity constant "K" for 1 g. of fresh leaf material.

RESULTS

Table 1 shows that Wisconsin-Havana Seed tobacco responded to virus infection with an increase in total nitrogen content in the diseased leaves. However, the virus concentration in extracts of the nitrogen-deficient plants was nearly normal. The plants grown on poor soil were less than half the size of those grown on an average greenhouse soil. Spencer's (16) observation that tobacco plants grown on a nearly nitrogen-free sand contained only 1/30 the virus content of plants grown in soil does not necessarily conflict with our results. His nitrogen-deficient plants received less than 10 per cent of the amount of soluble nitrogen fed to the medium-nitrogen series, and made only 1/9 the normal amount of growth. Under such conditions of severe nitrogen starvation he was able actually to reduce the virus concentration after the first systemic spread.

The response of T.I. 448A tobacco grown on good soil was erratic. The average of 6 experiments shows a slight increase in total nitrogen of the diseased plants as compared to the healthy controls. The decreased nitrogen content of the diseased plants grown on poor soil indicates that the effect of virus infection on the resistant T.I. 448A tobacco is influenced by the level of nitrogen supply. Assays on T.I. 448A plants showed very low levels of virus concentration, even at the stage of maximum virus concentration, and in some instances the amount was too small to show up in a 10^{-1} dilution.

TABLE 1.—*The influence of common-mosaic virus on the total nitrogen content of susceptible and resistant tobacco plants cultured at different levels of nitrogen supply. Percentage of nitrogen based on dry weight of tissue*

No. of experiment	Days from inoc. to analysis	Poor soil			Good soil			Good soil plus nitrogen		
		Healthy plants	Diseased plants		Healthy plants	Diseased plants		Healthy plants	Diseased plants	
		Nitrogen	Nitrogen	Lesions per test leaf	Nitrogen	Nitrogen	Lesions per test leaf	Nitrogen	Nitrogen	Lesions per test leaf
	No.	*Per cent*	*Per cent*	*No.*	*Per cent*	*Per cent*	*No.*	*Per cent*	*Per cent*	*No.*
	Wisconsin-Havana Seed Tobacco (Susceptible)									
I		....	....	....	3.70	4.35	28.9[a]	5.75	6.39	25.7[a]
II	16	....	....	....	4.57	5.35	15.1	5.81	6.57	9.6
II	26	....	....	....	3.39	4.31	35.9	4.74	5.38	43.1
IV	23	....	....	....	3.96	5.08	32.7	....	....	....
V	24	3.74	4.35	19.4[a]	3.24	4.50	25.1	5.92	6.56	25.5
	T.I. 448A Tobacco (Resistant)									
II	16	....	....	....	5.15	4.98	.32[a]	4.74	5.38	.46[a]
II	26	....	....	....	3.60	3.37	4.3	4.48	5.00	29.7
III	9	3.43	3.07	3.8[a]	4.81	4.87	7.3	....	....	....
III	21	3.62	2.61	0.0	3.16	3.79	0.0	....	....	....
III	32	2.98	2.98	0.0	2.78	2.79	0.0	....	....	....
IV	23	....	....	....	5.13	5.55	48.8	....	....	....

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[a] Virus extracts from Wisconsin-Havana Seed tobacco were diluted to 10^{-3} and those from T.I. 448A tobacco were diluted to 10^{-1} in phosphate buffer at pH 7.0. Each sample was then wiped on 30 bean leaves.

TABLE 2.—*The influence of common-mosaic virus on the nitrogen fractions and virus-protein in susceptible and resistant tobacco cultured at two levels of nitrogen supply. Percentages of nitrogen based on dry weight of tissue. Analyses made 21 days after inoculation*

Plants	Good soil					Good soil plus nitrogen				
	A	B	C	D	E	A	B	C	D	E
	Nitrogen (total)	Soluble protein nitrogen	Non-protein nitrogen	Virus protein nitrogen	Lesions per test leaf	Nitrogen (total)	Soluble protein nitrogen	Non-protein nitrogen	Virus protein nitrogen	Lesions per test leaf
	Per cent	*Per cent*	*Per cent*	*Per cent*	*No.*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*No.*
Wisconsin-Havana Seed Tobacco (Susceptible)										
Healthy	3.24	.109	.261	....	....	5.92	.172	1.36	....	
Diseased	4.50	.168	.320	.091	20.1[a]	6.56	.264	1.33	.107	33.7[a]
T.I. 448A Tobacco (Resistant)										
Healthy	3.60	.099	.565	....	....	4.48	.095	.937	....	....
Diseased	3.37	.092	.498	[b]	4.3[a]	5.00	.106	1.275	[b]	29.7[a]

[a] Fresh-leaf samples from infected Wisconsin Havana Seed tobacco were diluted to 10^{-3} and those from T.I. 448A tobacco were diluted to 10^{-1} in phosphate buffer at pH 7.0. Dilutions based on grams fresh weight per cc. of buffer. Tissues finely pulped in mortar, then brought to final dilution. Each sample wiped on 30 bean leaves.

[b] Virus protein concentration too low to measure by chemical methods.

A more detailed analysis of the effect of virus infection on the nitrogen metabolism of susceptible and resistant strains of tobacco is provided by the data in table 2. All analyses were calculated on the dry-weight basis. However, the dilutions used in making virus assays were calculated on the fresh-weight basis.

The virus concentration in extracts of T.I. 448A tobacco leaves was only 1 per cent of the virus concentration in extracts of Wisconsin-Havana Seed tobacco as determined by biological assay on bean leaves. The virus-protein content in T.I. 448A tobacco was too low to be determined accurately by the chemical method. Virus-protein nitrogen, as determined by the isoelectric precipitation method, is recorded in table 2, column D.

Wisconsin-Havana Seed tobacco plants infected with virus developed a relatively high concentration of the abnormal virus nucleoprotein. Although analysis of these plants showed an increase in the soluble-protein fraction, the increase did not equal the amount of abnormal virus protein included in the soluble-protein fraction. This means that the plant produced a reduced amount of normal protein in the presence of the virus protein. The plants grown on an increased nitrogen supply showed the same, though a less marked, replacement of part of the normal protein by virus protein. The changes in nonprotein nitrogen were more pronounced in the plants grown on an average nitrogen level than in those grown on a high nitrogen level.

The resistant strain T.I. 448A showed a much less marked response to virus infection than did the susceptible variety. Our results confirm those previously reported by Martin, Balls, and McKinney (11), who found that virus infection caused a decrease in the total-nitrogen content of a mosaic-resistant tobacco. The soluble-protein nitrogen and nonprotein nitrogen of T.I. 448A tobacco seem to parallel the total-nitrogen content of the plant and are not noticeably altered by the presence of mosaic virus. The virus content of this resistant strain never reaches a high level, and the plants do not develop mosaic symptoms.

A study was made of the effect of virus infection on the chlorophyll content and on the activity of some of the enzymes of susceptible and resistant tobacco plants. The plants were collected 23 days after inoculation, when the mosaic symptoms in Wisconsin-Havana Seed tobacco were fully developed. The samples included all of the mosaic-mottled leaves. Comparable tissues were collected from the healthy control plants. All determinations were made on freshly ground leaf tissue that had been freed of petioles and midribs. Owing to conditions not under the control of the authors, it was not possible to divide the samples on the basis of the several stages of tissue development. The data are arranged in table 3.

The decrease in chlorophyll and chlorophyllase observed in the diseased Wisconsin-Havana Seed tobacco agrees with the results of Peterson and McKinney (12).

The resistant strain, T.I. 448A tobacco, showed no changes in the concentration of leaf chlorophyll nor in the activity of the leaf chlorophyllase.

Oxidase and catalase were decreased by the virus in the susceptible and the resistant plants. However, in the case of peroxidase, there was a decrease in the susceptible plants and an increase in the resistant plants. In general, the changes in the enzyme contents were less in the infected resistant plants than they were in the infected susceptible plants.

Guthrie (6), using the iodometric method, found in mosaic-diseased tobacco 3 times as much oxidase as in healthy tobacco. Dunlap (5) made a point of separating the young from the mature tissues in his samples of tobacco tissue, with the result that he found a higher than normal respiration rate in the mature diseased tissue. Guthrie (6) may have used young leaves or young plants in his studies, but this point is not clear. If the writers had been in a position to study tissues in different stages of development, it is quite possible that their results would have harmonized with those reported by Guthrie and by Dunlap.

TABLE 3.—*The influence of common-mosaic virus on the chlorophyll and the enzyme content of susceptible and resistant tobacco plants. Determinations made 23 days after inoculation*

Plants	Chlorophyll	Chlorophyllase	Oxidase	Peroxidase	Catalase
Wisconsin-Havana Seed Tobacco (Susceptible)					
	Mm.	*Mm.*	*Cc.*	*"K"/g.*	*"K"/g.*
Healthy	20.0[a]	20.00[a]	3.580[b]	0.845[c]	1.600[c]
Diseased	29.0	27.60	2.400	0.630	0.765
T.I. 448A Tobacco (Resistant)					
Healthy	22.5	18.00	3.000	0.890	1.345
Diseased	22.4	18.00	2.250	1.080	1.145

[a] Depth of solutions of equal concentration required to match standard in colorimeter.

[b] Oxidase activity expressed in cc. of N/100 solution of sodium thiosulphate per g. of fresh material with a reaction time of 1 hour at 30° C.

[c] Peroxidase and catalase activities expressed as the reaction velocity constant "K" per g. of fresh material.

DISCUSSION

In Wisconsin-Havana Seed tobacco, virus multiplied rapidly during the first 10 days or 2 weeks following inoculation (10, 11), and when the mosaic symptoms were fully developed the virus-protein concentration of the leaves amounted to 50 per cent or more of the soluble-protein nitrogen and approximately 2 per cent of the total nitrogen of the diseased leaves.

The increase in total nitrogen of susceptible tobacco plants infected with common-mosaic virus was confirmed by our present study. Wisconsin-Havana Seed tobacco infected with common mosaic showed an increase in total-nitrogen content, which was much greater than the actual increase in virus protein. This would seem to indicate that mosaic infection stimulated the plant to synthesize an abnormally high amount of protein, the major portion of which was water-insoluble tissue protein, only a small fraction consisting of the virus protein itself.

Wisconsin-Havana Seed tobacco, grown on a high nitrogen level, was able to synthesize nearly the normal amount of water-soluble protein in addition to the large quantity of abnormal virus protein present, but plants grown on a lower nitrogen level showed a greater replacement of soluble protein by virus protein.

The mosaic-resistant strain (T.I. 448A) showed a much different response to virus infection. A decrease in total nitrogen was observed in plants grown with a low nitrogen supply. The virus concentration in such plants was very low and the leaves formed after inoculation contained little or no virus. Mosaic-resistant plants grown on the higher levels of nitrogen supply showed no decrease in total nitrogen, and in some instances an actual increase was observed. The virus reached a higher concentration, and the leaves formed subsequent to inoculation escaped infection at a later stage in the growth of the plants. It is apparent that the resistance of such plants to mosaic virus is modified to a limited extent by the level of nitrogen nutrition.

The effect of mosaic virus on the total nitrogen content of susceptible and resistant strains of tobacco has a commercial significance because with the more important types of tobacco a low nitrogen content is very desirable.

Evidence that viruses produce fundamental changes in the carbohydrates and enzymes of plants has accumulated from various sources (1, 5, 12). Dunlap (5) observed that, although mosaic virus caused an increase in the total nitrogen of tobacco, there was a marked decrease in carbohydrates. Respiration was higher in young tobacco plants infected with mosaic, but in older plants the respiration was decreased. The effect of age on the activities of various enzyme systems in mosaic-diseased tobacco may explain the increased oxidase activity observed by Guthrie (6) in mosaic-diseased tobacco leaves. Our results, derived from the total mosaic-diseased tissue from plants 23 days after inoculation, showed an actual decrease in oxidase activity as compared with healthy controls. We also observed decreases in the catalase, peroxidase, and chlorophyllase in the infected susceptible tobacco. The infected resistant tobacco showed a decrease in oxidase and catalase nearly equivalent to that observed in the infected susceptible variety, whereas the virus concentration in the resistant plants was only about 1 per cent that of the susceptible plants. This low concentration of virus was sufficient to produce a measurable disturbance in the metabolism of the plant, but it was not enough to induce visible signs of disorder.

An outline of the interrelationship of viruses and environmental factors in the metabolism of plants has been discussed recently by Selman (14). He suggests that the primary effect of plant virus infection on the metabolism of the plant is an abnormal protein synthesis, and that the presence of the virus protein affects the permeability of the tissues, transpiration, and the carbohydrate metabolism.

The exact sequence of the changes induced as a result of virus infection cannot be determined from existing data, but from the writers' (9) studies on the perennial pepper plant (*Capsicum frutescens* L.) it is reasonable to believe that the virus soon initiates reactions which result in one or more products that are destructive to many kinds of plants. In the case of the perennial pepper inoculated with *Nicotiana Virus 1* and cultured near 32° C., these products appear to move some distance from the sites of detectable virus, destroying the chlorophyll in mature leaves and occasionally inducing mosaic mottling in the young leaves.

Mosaic mottling in tobacco may be caused directly by the products of a deranged metabolism that is induced directly by the virus. However, in mosaic-susceptible tobacco, such as Wisconsin-Havana Seed, active virus is very closely associated with the chlorotic areas.

SUMMARY

A study was made of the effect of common-mosaic virus on the nitrogen content of a mosaic-susceptible tobacco (Wisconsin-Havana Seed) and a mosaic-resistant tobacco (T.I. 448A).

Virus infection caused a marked increase in the total-nitrogen content of susceptible tobacco grown under conditions of low, medium, and high nitrogen nutrition. The resistant tobacco, when grown with a reduced nitrogen supply, showed a decrease in total nitrogen in the virus-infected plants, but when the plants were grown with a more adequate nitrogen supply, they showed no decrease in total-nitrogen content.

Mosaic-diseased leaves of Wisconsin-Havana Seed tobacco contained 30 per cent less chlorophyll and had a lower chlorophyllase activity than healthy leaves. There was no change in the chlorophyll or chlorophyllase of virus-infected leaves of tobacco T.I. 448A.

The oxidase activity of both the resistant and susceptible strains of tobacco was decreased by mosaic virus, and, while the changes in the enzyme activities caused by virus infection were slightly less in the resistant than in the susceptible variety, it is evident that a very small amount of virus nucleoprotein was sufficient to produce measurable alterations in the metabolism of the mosaic-resistant tobacco.

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THE DOWNY MILDEW DISEASE OF OATS, CAUSED BY SCLEROSPORA MACROSPORA[1, 2]

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INTRODUCTION

The genus *Sclerospora* has attracted increasing attention during recent years because of the destructive parasitism of its species on a number of important gramineous host plants. Although several species of the genus are know to attack members of the grass family, only two have been found in the United States. These are *Sclerospora graminicola* (Sacc.) Schroet. and *S. macrospora* Sacc. Both are worldwide in their distribution and both occur in temperate and tropical regions. Our present knowledge of these fungi, particularly the latter, is scanty, and additional information relative to the appearance of either of these fungi in new localities or on additional host plants is of considerable importance to agriculture. Accordingly, when *S. macrospora* was found for the first time in Mississippi and for the first time on oats in the United States, it seemed necessary to investigate its potential destructive activity and to appraise the possibility of it becoming a menace to the oat crop, now rapidly attaining a position of importance in the Delta area along the Mississippi River, where this infestation was found.

The purpose of this paper is, therefore, to describe the symptoms of oat downy mildew, compare the causal organism with other species of the genus *Sclerospora*, and report results of preliminary studies on certain phases of its life cycle.

THE DISEASE

Distribution in Mississippi

The disease was discovered in the spring of 1939 by the senior writer (12) on oat specimens collected by a grower and submitted for diagnosis. They were collected in the vicinity of Indianola, Sunflower County, Mississippi, in the Yazoo-Mississippi Delta. The plants were in an advance stage of development, and, a large part of the crop having already been harvested, no extensive survey for determining the extent of the trouble was possible for that year. A limited search, however, showed the mildew present in numerous fields in the immediate neighborhood of Indianola and Inverness. The infested fields were sown to locally grown seed, which indicated that the disease probably had been present for some years, or at least that it was not of recent introduction. A brief survey in the spring of 1940 revealed its

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[3] Deceased, May 10, 1941.

presence in the same fields in which it had occurred in 1939. In a few instances the fields had been resown to the same crop. The disease also was found to be of common occurrence in the adjacent counties of Washington, Sharkey, and Leflore. It was later found in oat fields at the Mississippi Agricultural Experiment Station, State College, indicating that the disease was not confined to the Delta region where it was first observed. The disease seemed to be most severe in low fields that, at some time during the growing season, had passed through a period of flooding. In such areas it was not uncommon to find nearly all plants showing infection. In other fields, not subjected to flooding, affected plants were found distributed over the entire area, although not in epiphytotic proportions.

Sclerospora macrospora was found in the spring of 1940 parasitizing a few volunteer wheat plants growing in an oat field near Leland. Although a number of wheat fields were carefully examined, this was the only instance in which the fungus was found in Mississippi on any host other than oats. It is possible, however, that a more extensive survey would reveal its presence on other grain crops and on a number of wild grasses, such for example, as *Bromus commutatus* Schrad. (cheat grass), commonly occurring in oat fields, and according to Weston (29), prevalent and generally infected in the vicinity of Tennessee and Kentucky wheatfields.

Distribution in Other Countries

Although the report (12) of the disease on oats in Mississippi constituted the first record of the occurrence of *Sclerospora macrospora* on that host in the United States, it had previously been recorded on oats from New South Wales (13, 14, 15), Italy (16, 17, 18, 19) and France (1). The fungus was reported in the United States in 1921 by Weston (29) on wheat and cheat grass in Kentucky and Tennessee, and was reported the same year by Mackie (10) on wheat in California.

Sclerospora macrospora was first described by Saccardo (24) in 1890 on foxtail grass (*Alopecurus* sp.) from Caronby, Australia. Noble (14) states that photographic records indicate that the disease was present on wheat in Queanbeyan, Australia, as early as 1891. Traverso (27), in 1924, reported that spikes of grain from the Cryptogamic Laboratory in Pavia showed that the disease had been present in Italy since 1873. The disease has been reported on wheat and oats from the United States (12, 29), Italy (16, 17, 18, 19, 22, 25, 26, 28), France (1, 2), and Australia (6); on rice from Italy (4, 5, 8), Japan (30), and Australia (15); on corn from Italy (7, 9), and Australia (14); on barley from the United States (10), and Italy (25, 28); and on rye from Australia (14, 15) and Bulgaria (3). In addition, it has been collected on various wild grasses from all the above-mentioned countries and from Germany (11).

Following is a list of host plants on which the fungus, *Sclerospora macrospora*, is known to occur: *Agropyron repens* (L.) Beauv., *Agrostis alba* L., *Alopecurus* sp. L., *A. agrestis* L., *Avena fatua* L., *A. sativa* L., *Bromus com-*

mutatis Schrad., *Cyperus articulatus* L., *Eragrostis major* Host, *Festuca elatior* L., *Glyceria maritima* Wahlb., *Holcus mollis* L., *Hordeum sativum* Pers., *Lollium perenne* L., *L. temulentum* L., *Oryza sativa* L., *Panicum antidotale, Phalaris arundinacea* L., *P. canariensis* L., *P. coerulescens, Phragmitis communis* Trin., *Secale cereale* L., *Syntherisma sanguinale* Dulac, *Triticum sativum* L., and *Zea mays* L.

Symptoms on Oats

Many of the affected plants had a tendency to be stiff and were characterized by an upright leaf habit. Such plants showed no internodal shortening; the upright deformed heads frequently stood above the more drooping, heavier panicles of unaffected plants. In some cases the uppermost leaves were curled and twisted about the poorly developed heads so that only small portions of the latter were visible (Fig. 1, A). Some plants produced a rachis of normal length, although so curled and twisted that it appeared much shorter than usual. A rachis of this type usually produced a small cluster of sterile spikelets at its tip (Fig. 1, B). In other cases the rachis was very much shortened and reduced to a small compact cluster of frayed and tangled spikelets (Fig. 1, C). Some plants exhibited a very peculiar deformation of the fruiting panicle. The rachis was variously coiled or much elongated and produced only a few sterile structures resembling spikelets. Instead of each spikelet bearing 2 seeds in close contact within the same glumes, there was a single seed-like structure that enveloped the rachilla. The latter, greatly enlarged and elongated beyond the tips of the

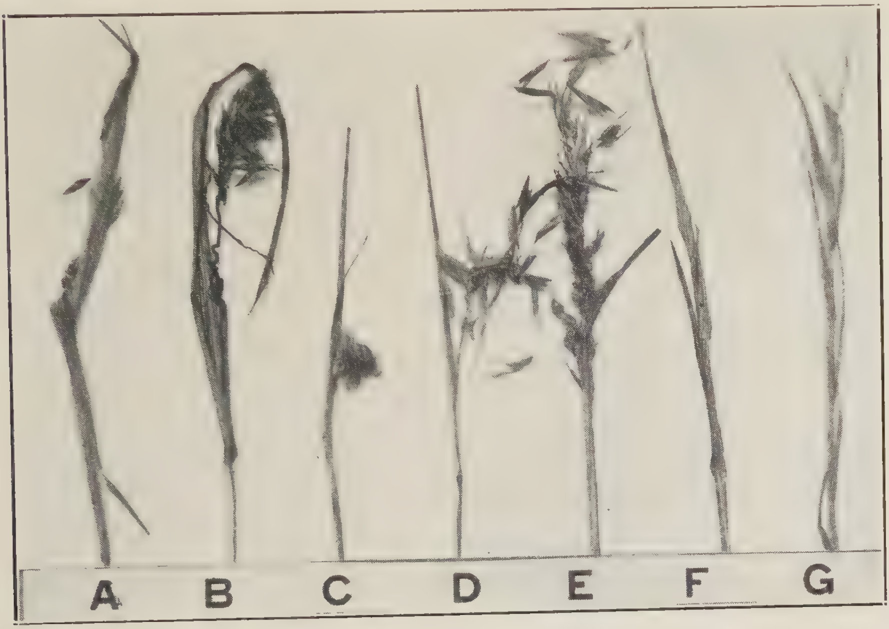

FIG. 1. Oat heads deformed by *Sclerospora macrospora.*

glumes, formed a node from which derived a second seed-like structure, very similar to the first, but lacking the enclosing glumes; the lemmas constituted the outer covering (Fig. 1, D). In some cases the rachilla, continuing to elongate, formed additional nodes sometimes bearing leaf-like structures. In other cases additional sterile seed-like bodies evolved. The rachilla frequently was of normal length and diameter, but became twisted about the rachis, giving a compact, elongated, head-like appearance to the fruiting panicle (Fig. 1, E).

In certain specimens of the type characterized by Fig. 1, D, the rachis was elongated as much as 4 or 5 inches between the sterile spikelets, or the sterile seed-like structures replacing them. The resulting panicle exhibited a scattered drooping aspect. Frequently the sterile seed-like structures were markedly elongate and somewhat curved. Occasionally, a single panicle showed all of these types of distortion; in other specimens only a single type occurred. In some specimens a single stool produced both normal and diseased heads, though this was unusual.

Many plants failed to develop heads and some apparently died early. The leaves of such plants were short, stiff, rather fleshy, and remained upright instead of curling downward normally (Fig. 1, F–G). On such plants the internodes usually were shortened and the tillers excessively numerous. Reddish-brown streaks and small ruptured areas were abundant on such leaves from the bases to the tips. Such lesions were much less common on plants producing deformed heads. These dwarfed, bunchy, nonfruiting plants were found scattered among those of normal height. Frequently, a stool was found with a number of culms of normal height and variously distorted. These stools also produced many bunchy, leafy branches from the base, some of which attained a height of only 4 or 5 inches.

Oospores were found in all diseased heads. They were closely invested with a heavy, persistent oogonial wall and were most abundant in the leaves, leaf-sheaths, rachis, rachilla, glumes, and other aboveground parts of the plants.

The disease was easily diagnosed in the oat plant on reaching the heading stage. By holding an infected leaf between the eye and the sun, with the aid of a hand lens, the oospores appeared as bright, translucent golden dots. They were usually grouped in rows between the vascular bundles, or in the bundles themselves, and were sometimes found scattered uniformly through the mesophyll tissues between the veins.

Symptoms on Wheat

The disease has been observed on a few wheat plants found in an infested field of oats. These plants were heading, much distorted, and sterile. The rachis was frequently so elongated as to widely separate the spikelets, especially toward the base, the head thereby losing its compact appearance. The rachis sometimes bent back on itself causing the head to form a circle or compact knot. The wheat found infected was bearded, the awns being much curled and distorted. Such heads, therefore, were very conspicuous. Oospores were abundant in leaves, glumes, and awns.

The Causal Organism

The conidial stage of *Sclerospora macrospora* has not been found occurring in nature. The mycelium was intercellular and particularly abundant in the region of the vascular bundles. Haustoria, in the sense of those occurring commonly in the Peronosporaceae, were apparently lacking. Knobbed or wart-like outgrowths, however, were frequently observed, especially in the neighborhood of bast cells or pressing closely against the walls of nonpenetrated sieve tubes. From the accumulation of mycelium along the vascular bundles single strands were observed extending out between the cells of the parenchyma. Magnus (11) reported that *S. kriegeriana* (shown by Saccardo to be merely a synonym of *S. macrospora*) on *Phalaris arundinacea* L. produced hyphal branches that extended to the stomata where they gave rise to side outgrowths. These pushed out as short papillae. Magnus suggested that these outgrowths possibly represented rudimentary conidiophores, capable, under certain conditions, of developing further and becoming functional. These structures have not yet been observed on infected oat plants.

Oogonia and antheridia developed abundantly on the hyphae that were parallel to the vascular bundles. The antheridia developed on side branches in the vicinity of the oogonia. It was evident, however, that the resting spore, as observed in infected oat plants, consisted of the mature oospore closely invested in the thick, persistent oogonial wall. This wall frequently showed remains of the oogonial stalk cell, and, in young resting spores, remains of the antheridium sometimes may be observed attached at some point on the side or apex. When fully mature the oogonia were quite distinctive. The oogonial wall was generally smooth, though sometimes, when removed from the host plant, it exhibited roughened areas caused by fragments of the host cells against which it was closely appressed and adherent. Sometimes a portion of the outer oogonial wall exhibited knobs or projections which conformed closely to the outline of the pits in the walls of the bast fibers or phloem sieve tubes against which they were appressed. The pale-yellow oogonial wall usually very closely invested the single oospore, though narrow lumina frequently were observed between the two walls. Where there was a thickened place in the oogonial wall, as sometimes occurred, it seemed to be caused by a natural thickening; probably induced by unequal pressure or irregularity in the intercellular space in which it was formed. It was not caused by an overlapping or folding, as was the case for *Sclerospora graminicola* (Fig. 2, B).

The average thickness of the oogonial wall of 543 oospores from oats, wheat, corn, and barley was 4.2 μ. When measured along the long diameter of the oospore, the average thickness of the wall was 4.4 μ as compared with 4.1 μ for the short diameter. The reason for this difference was caused by the partial persistence of the old oogonial stalk cell. In a few cases the thickness at this point was as much as 10 μ.

The oospores varied in shape from spherical to oblong. The walls, always smooth, varied in thickness from 2 to 4 μ, and lacked the yellow color of the oogonial wall. The oospore contents were yellowish-gray and densely granular. Sometimes lighter areas occurred in which small oil-like droplets were observed. In Table 1 are presented measurements of oospores from a number of host plants from various localities. Only average diameters of oospores are considered significant, since observations have shown this to be the most constant factor. The enclosing oogonial wall was disregarded in all cases in making measurements, since its shape varied widely because of the variation in contour of the intercellular spaces in which the spores were formed. The number of measurements recorded varied for different collections, ranging from 900 on cheat grass from Tennessee and Kentucky to 50

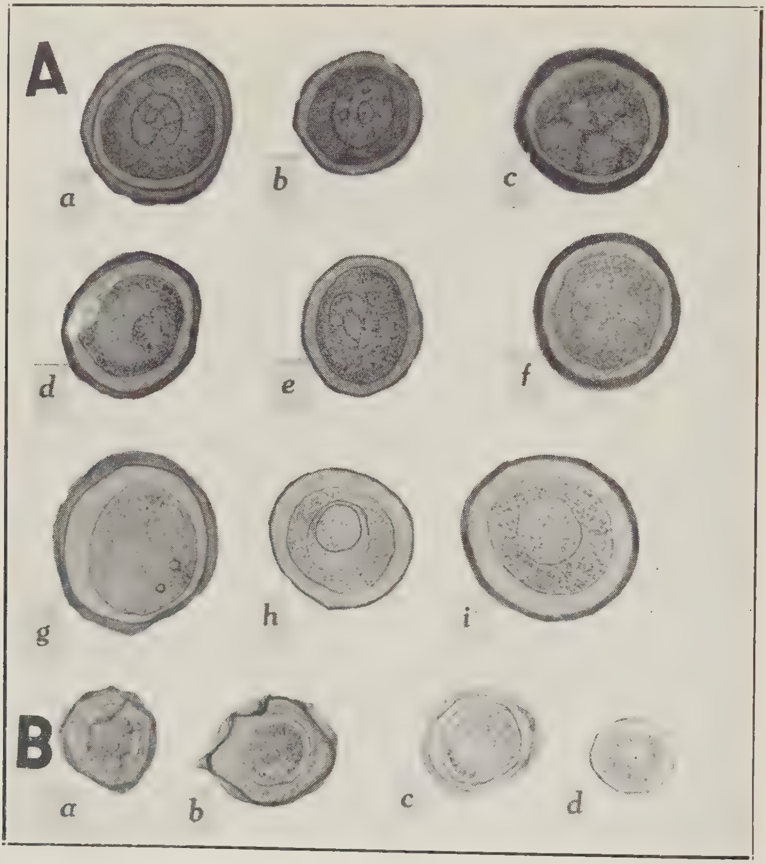

FIG. 2. A. Oospores of *Sclerospora macrospora* from *Avena sativa* L.; *a–c*, collected at Indianola, Mississippi; *b*, oogonial wall removed; *d–f*, collected at Bologna, Italy; *e*, oogonial wall removed; *g–i*, collected in New South Wales, Australia; *h*, oogonial wall removed. B. Oospores of *S. graminicola* from *Pennisetum typhoideum* L. Rich. collected at Poona, India; *d*, oogonial wall removed. Camera lucida drawings. ×375.

on a number of others in which only a limited amount of material was available. Some of the measurements were made by the writers and some by W. H. Weston.

Two collections from barley are presented in the table, one from California, collected by J. E. Coke, and one from Italy, collected by Vittorio Peglion. The Italian collection averaged larger both in average diameter and in mode than did the one from California. The total range, however, was very similar for both.

TABLE 1.—*Comparative diameter of the oospores of Sclerospora macrospora and S. graminicola collected by various investigators in several localities*

Host	Locality	Collector	Number of oospores measured	Average diameter of oospores in microns	Range in microns	Mode in microns
		Sclerospora macrospora				
Oats	Mississippi	L. E. Miles	335	55.6	34.6–74.9	54–56
Oats	Yanco, New South Wales	R. J. Noble	100	58.6	40.6–73.8	54–56
Oats	Sydney, New South Wales	R. J. Noble	100	61.8	49.0–79.0	56–58
Wheat	Kentucky and Tennessee	W. H. Weston	612	62.5	38.2–88.2	62–64
Wheat	Delaware	J. F. Adams	168	60.9	50.9–78.0	60–62
Wheat	Assencini, Italy	Cavara	50	60.1	45.0–72.0	62–64
Wheat	Piemonte, Italy	....	50	65.6	43.2–68.4	54–56
Wheat	Samoggia, Italy	G. Goidanich	50	64.3	52.2–77.4	62–64
Wheat	Sardigna, Italy	Briosi	50	58.6	49.0–72.0	54–58
Wheat	Bologna, Italy	V. Peglion	100	53.6	45.0–64.8	54–56
Wheat	Padova, Italy	Saccardo	50	62.9	52.9–75.6	62–64
Barley	California	J. E. Coke	100	58.9	47.0–71.2	56–58
Barley	Bologna, Italy	V. Peglion	100	63.0	45.0–72.0	62–64
Corn	Bologna, Italy	V. Peglion	100	53.9	36.0–69.4	54–56
Rice	Taki-no-saki, Japan	G. Yamada	100	50.8	35.0–64.0	50–52
Rice	Morioka, Japan	G. Yamada	200	47.9	32.0–66.0	50–54
Avena fatua	Parkes, New South Wales	R. J. Noble	175	48.0	31.0–60.0	46–48
Bromus commutatus	Tennessee	W. H. Weston	900	60.1	32.0–84.0	62–64
Eragrostis major	Condobolin, New South Wales	R. J. Noble	50	62.3	56.1–72.7	62–64
Syntherisma sanguinale	Morioka, Japan	G. Yamada	100	44.9	30.3–55.4	44–46
		Sclerospora graminicola				
Pennisetum typhoideum	Poona, India	B. N. Uppal	200	30.4	21.6–39.6	32–34

Only one collection from corn was measured. This was made by Peglion in Bologna, Italy. It is worthy of note that the measurements on this host were practically identical with those on wheat, collected in the same locality in the same year by the same collector.

Two collections on rice from Japan, from two different localities, namely, Taki-no-saki and Morioka, were measured. These agreed quite closely but were smaller than the measurements from either oats, wheat, barley, or corn. Only one other collection of *Sclerospora macrospora* from Japan was available, and it was on a grass, *Syntherisma sanguinale,* collected by G. Yamada, in Morioka, Japan, in 1911. The spores in this instance were even smaller than on rice, and were the smallest observed. The average diameter of the oospores was only 44.9 μ, the range in diameter was from 30.3 to 55.4 μ, and the mode was 44 to 46 μ.

A collection of oospores on *Avena fatua,* a wild oat, collected near Parkes, New South Wales, by R. J. Noble in 1926, was only slightly larger, being about midway between the one on *Syntherisma sanguinale* and those of two collections on rice from Japan. It is interesting to note that a collection on *Eragrostis major* made at Condobolin, New South Wales, by R. J. Noble in 1936, had considerably larger spores, agreeing rather closely with the sizes of those on wheat and on cheat from Tennessee and Kentucky.

A collection of *Sclerospora graminicola* on *Pennisetum typhoideum* from Poona, India, collected by B. N. Uppal, in 1934, was measured and is presented in the table in order to show the much smaller spore sizes for the latter species as compared with *S. macrospora.* The average diameter of 200 spores of *S. graminicola* was only 30.4 μ, the range in diameter was from 21.6 to 39.6 μ, and the mode was from 32 to 34 μ. It will be noted that the average diameter of the oospore of this latter species was essentially the same as the diameter of the smallest oospore measured on *Syntherisma sanguinale*, which had the smallest spores of any collection of *S. macrospora* measured.

The average diameter of 335 oospores from oats collected in Mississippi in 1939 and 1940 was 55.6 μ, while 100 spores from a collection on the same host from Yanco, New South Wales, made by R. J. Noble, in 1924, averaged 58.6 μ and 100 spores from another oat collection from Sydney, Australia, averaged still larger, namely 61.8 μ. The mode for the average diameter measurements for the collections from Mississippi and for the one from New South Wales, Australia, was 54–56 μ, while that for the other collection from Australia was 2 μ larger.

The average diameter of 612 oospores from wheat from Tennessee and Kentucky, collected by W. H. Weston, was 62.5 μ; that of 168 oospores from wheat from Delaware, collected by J. F. Adams, was 60.9 μ; and the average diameter of 400 spores from 7 different collections from various points in Italy, made from different localities and in different years by different collectors, was 59.6 μ. The mode for the Tennessee and Kentucky collections was from 62 to 64 μ, while that from Italy was 58 to 61 μ.

These measurements tend to exhibit a very considerable degree of similarity in spore sizes for all collections from the United States, Italy, and Australia, regardless of the host plant, except for the single collection on *Avena fatua* from New South Wales. This seems to fall into another group composed of the specimens on rice and *Syntherisma sanguinale* from Japan. It is unfortunate in this case that no collections of the fungus on rice from Italy were available for measurement. Table 2 presents a tabulation of

TABLE 2.—*Tabulation of frequency distribution of oospore size of Sclerospora macrospora on various host plants from several localities*

Range in diameter of oospores in microns	Oats		Wheat			Cheat	Rice		*Syntherisma sanguinale*	*Avena fatua*
	New South Wales	Mississippi	Kentucky & Tennessee	Delaware	Italy	Kentucky & Tennessee	Takinosaki, Japan	Morioka, Japan	Japan	New South Wales
	100 spores	335 spores	612 spores	168 spores	355 spores	900 spores	100 spores	200 spores	100 spores	175 spores
30–31.9	......	......	......	......	......	......	......	......	2	1
32–33.9	......	......	......	......	......	2	......	2	2	1
34–35.9	......	1	......	......	......	1	1	......	5	1
36–37.9	......	1	2	......	......	......	......	3	2	2
38–39.9	......	1	1	......	......	3	......	2	7	8
40–41.9	1	8	......	......	......	6	1	4	10	11
42–43.9	2	4	3	......	2	8	9	8	9	12
44–45.9	3	23	8	......	4	6	13	14	15[a]	21
46–47.9	2	13	14	......	10	27	16	22	13	34[a]
48–49.9	4	21	14	......	10	42	15	29	13	27
50–51.9	7	18	16	2	22	64	18[a]	37[a]	12	23
52–53.9	4	20	30	14	18	50	12	21	8	10
54–55.9	15[a]	75[a]	37	18	37	52	7	15	3	8
56–57.9	8	29	42	28	36	81	4	13	......	5
58–59.9	10	26	62	28	41	98	1	10	......	4
60–61.9	10	29	64[a]	30[a]	45	103	2	2	......	7
62–63.9	11	33	54	17	57[a]	105[a]	1	6	......	......
64–65.9	6	6	59	17	26	82	......	......	......	......
66–67.9	8	10	63	6	15	55	......	......	......	......
68–69.9	3	10	46	2	15	52	......	......	......	......
70–71.9	2	4	30	3	13	39	......	......	......	......
72–73.9	4	2	21	......	1	25	......	......	......	......
74–75.9	......	1	20	......	2	9	......	......	......	......
76–77.9	......	......	12	1	1	5	......	......	......	......
78–79.9	......	......	5	......	......	2	......	......	......	......
80–81.9	......	......	4	......	......	4	......	......	......	......
82–83.9	......	......	2	......	......	2	......	......	......	......
84–85.9	......	......	2	......	......	1		......	......	......
86–87.9	......	......	2	......	......	......	......	......	......	......

[a] Mode, or range within which the largest number of oospores were found.

frequency distribution of oospore diameters for several of the above collections and shows this grouping in a more graphic form.

DISSEMINATION AND PERSISTENCE OF THE PARASITE

Since a conidial stage has not been found, dissemination and persistence of the causal organism must occur most frequently through the resting spores. There is a slight chance that the mycelium might reach the seed and lie dormant therein, although plants are usually so severely diseased as to produce no viable seeds. It seems improbable that wind is a very important factor in the dissemination of the oospores since, unlike *Sclerospora graminicola*, the invaded tissues are rarely shredded. It is possible that eroding soil, containing oospores, may be a means of distribution.

There is little doubt that the fungus persists in the soil of infested fields through the agency of the oospore, invested and protected as it is by the thick, hard, persistent oogonial wall. The writers have not yet observed the germination of the oospore, and this has been the experience of most others who have worked with this fungus, or for that matter with other species of the genus *Sclerospora*. Both laboratory and field observations, however, indicate that the oospores do undoubtedly germinate and bring about infection of the host plant.

Peglion (20, 21) reported germination of oospores of *Sclerospora macrospora* in 1914. The germination occurred by means of a large macroconidium produced on a short peduncle that issued through a split in the wall. The mature lemon-shape macroconidium bore a very prominent papilla and measured 75–80 × 55–60 μ. The contents of the conidium became differentiated into zoospores, though in rare instances the conidium germinated by means of a germ tube. Peyronel (23) reported similar results from a slightly different method. He observed germination of the oospores of *S. macrospora* by means of a conidium, or zoosporangium, and zoospores. This report is extremely interesting from a taxonomic as well as a practical viewpoint, since, if substantiated and confirmed as a general occurrence, it would necessitate removal of the species from the genus *Sclerospora* or, at least, a revision or redescription of the genus, since the present accepted method of germination for all members of the genus is by means of a germ tube issuing directly from the oospore.

Dissemination from one locality to another may occur through trash in the seed, consisting of leaf and glume fragments or other oosporic-bearing material, and by wind and erosion. Centrifugal separation has disclosed free oospores and minute fragments of oospore-bearing materials in seed lots that, from casual inspection, would have been considered well-cleaned. Moreover, some of the seed-like structures that replace the grain in the infested plant may very readily, and probably do, become mixed with the seed grain and might carry either spores or dormant mycelium and thereby establish an infestation in a new field. Further investigation is necessary to settle these points.

There are also other questions that require further elucidation. It has become rather generally accepted that a period of flooding of the host is a necessary condition to its infection through the oospores of *Sclerospora macrospora* (29). The writers, likewise, have found that the disease in Mississippi is much more frequent on land that has undergone a period of flooding. It has, however, been observed in numerous high and well-drained fields where flooding could scarcely have been possible. It is true that in such cases infected plants were evenly and sparsely scattered and that in such fields the disease did not occur in epiphytotic proportions. Coke (according to Mackie) stated (10) that the disease nearly destroyed a crop of barley in California in a region where rainfall was scanty and where at least one irrigation was necessary to bring the crop to proper maturity.

DISCUSSION AND CONCLUSION

At present the downy mildew of wheat and oats (*Sclerospora macrospora*), as it occurs in Mississippi, is not of great economic importance. In isolated instances and restricted areas, however, where the fields are low-lying and subject to submergence, or where drainage is poor, the losses locally may be quite considerable. It is becoming a common practice in Mississippi to grow oats on the ''buck shot'' (clay) type of land, especially in the Delta. Since cotton acreage reduction practices have come into general use, the smaller number of acres devoted to cotton are being selected from the more loamy types of soil, where conditions permit, since such soil can be more readily prepared for early planting, thereby leaving the heavier and wetter areas for oats and similar crops. Such ''buck shot'' soil does not require complete submergence to become thoroughly saturated with water for considerable periods of time. The increasing tendency to utilize such practices may render more serious this disease of the oat crop, which is rapidly growing in importance in the particular area of the State in which the trouble occurs. With wider dispersal of the spores of the causative organism and with increasingly heavy soil infestation from repeated cropping with a susceptible crop, heavy and widespread losses may occur under certain seasonal conditions especially favorable to the pathogen.

SUMMARY

This report comprises the first record of *Sclerospora macrospora* (downy mildew) on oats in the United States and the first record of the disease in the State of Mississippi.

Symptoms of the disease on oats are described and compared with symptoms on wheat. Affected oat plants are characterized by stiff, curled, and fleshy leaves. The rachis is usually short and twisted and produces few spikelets, most of which are sterile. Many diseased plants fail to produce heads and apparently die early in the season.

Spores are produced in all parts of the plant, except the roots, and are much more abundant in the leaves and glumes.

The average diameter of 335 oospores from oats collected in Mississippi in 1939 and 1940 was 55.6 microns.

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A COMPARISON OF DIPLODIA NATALENSIS FROM STAINED WOOD AND OTHER SOURCES

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INTRODUCTION

One of the important staining fungi on logs and lumber in the Southern States has been referred to *Diplodia natalensis* Evans (1, 8). Morphologically similar fungi have been reported as pathogens on plants, including cotton (3), citrus (6), Honey Dew melon (10), and strawberry (5). Stevens (7) referred the citrus form, *D. natalensis,* and the cotton form, *D. gossypina* Cooke, to the same perfect stage *Physalospora rhodina* (Berk. and Curt.) Cooke, and stated that this fungus appears to have a wide distribution geographically and in its host relationships. Cross inoculations (2, 9, 10) have proved the wide range of pathogenicity of the pycnidial forms of *P. rhodina* from a large number of hosts. None of this inoculation work, however, was done with isolates from stained logs or lumber.

Little fruiting of *Diplodia natalensis* was observed on stained lumber and logs from which this fungus was isolated, and, in an earlier paper (8), the author suggested that the inoculum of *D. natalensis* causing stain in logs and lumber largely came from other sources. To test this contention, isolates of *D. natalensis,* or similar fungi, were secured from cotton bolls, a tung root, a pear stem, and a citrus fruit, to compare with the isolates secured from stained wood as to certain cultural characteristics, spore size, wood-staining ability, and pathogenicity on orange fruits, cotton bolls, and the stems of tung seedlings.

THE ISOLATES, THEIR CULTURAL CHARACTERISTICS

In table 1 are listed pertinent data on the source of the isolates used, together with data on certain cultural characteristics.

Although among the isolates there was considerable variation in general cultural appearance when grown on malt agar at room temperature (25°–30° C.), no constant differences between isolates from different host groups were evident. All grew rapidly, 15 to 20 mm. in radius per day, at room temperature.

Each isolate was grown in duplicate on malt agar at about 37° C. (35.6°–38.9° C.), and all were found to grow at this temperature (Table 1). Rapid growth at temperatures above 35° C. was one of the characteristics previously reported (8) for the wood-staining *Diplodia natalensis.* At this high temperature all the isolates, except 2 from wood (W 5 and 6), produced a pink coloration in the mat and agar instead of the usual grayish-black shades produced at room temperatures. Pink chromogenesis of agar at 37° C. has been described for some forms of *Physalospora rhodina* (7, 9).

Spore measurements (Table 1) were each based on 20 exuded, bicellular, colored spores formed on malt agar at 25 to 30° C. Although there was considerable variation in average spore size, there was little indication of significant differences between isolates from wood and from other sources, and all fell well within the range for average spore size of different isolates listed by others (7, 9). All the isolates were similar in having longitudinal striations in the cell walls of the dark, bicellular spores and in producing hyaline, unicellular spores preceding or in mixture with the 2-cell spores. The average spore size of the isolates from wood was somewhat larger than previously reported (8) for a larger number of isolates from wood.

TABLE 1.—*The isolates of Diplodia used—source, type, growth rate, and spore size*

Designation	Host from which isolated	Locality and date of isolation	Type of isolate	Growth in 24 hours at 37° C. in mm.	Average spore size in microns
C1	Cotton	Louisiana 1939[a]	Mass	5.5	26.3 × 14.0
C2	Cotton	Mississippi 1938[b]	Monoconidial	7.0	26.4 × 14.1
C3	Cotton	Alabama 1939[b]	Mass	8.0	25.3 × 12.2
C4	Cotton	So. Carolina 1939[b]	Mass	6.5	26.0 × 12.8
C5	Cotton	Virginia 1939[b]	Mass	5.5	25.1 × 12.6
Cit	Citrus	Florida[c]	?	7.0	28.1 × 14.4
P	Pear	Louisiana 1936[a]	?	4.0	30.4 × 14.8
T	Tung	Louisiana 1936[a]	?	7.0	24.2 × 12.7
W1	Pine log	Louisiana 1937	Monoconidial	7.5	26.5 × 13.4
W2	Pine lumber	Louisiana 1937	Monoconidial	7.0	26.4 × 13.5
W3	Pine lumber	Louisiana 1937	Monoconidial	8.5	29.8 × 14.3
W4	Pine log	Louisiana 1937	Monoconidial	7.0	28.0 × 12.7
W5	Yel. poplar lumber	Louisiana 1937	Monoconidial	4.0	26.2 × 15.0
W6	Yel. poplar log	Louisiana 1937	Monoconidial	7.0	30.0 × 15.4
W7	Magnolia log	Louisiana 1937	Monoconidial	7.0	26.2 × 11.8
W8	Sweet-gum lumber	Louisiana 1937	Monoconidial	2.5	26.9 × 14.4

[a] Secured from Louisiana State University.
[b] Secured from the South Carolina Agricultural Experiment Station, Clemson.
[c] Secured from the Florida Agricultural Experiment Station, Citrus Experiment Station, Lake Alfred.

Due to the difficulty of separating *Physalospora rhodina*, the perfect stage of *Diplodia natalensis*, from certain other species of *Physalospora* on the basis of the morphology of the pycnidial forms and host relationships (7, 9), it is not certain that all the isolates from stained wood, that have the general characteristics of *D. natalensis*, are in reality *P. rhodina*. No perfect stages of the isolates used in this study were observed in culture nor have any been observed on stained logs and lumber. For convenience, the wood-staining forms are called *Diplodia natalensis* but with the realization that if complete life histories were known some isolates might have perfect stages other than *P. rhodina*.

WOOD-STAINING ABILITY

To test their wood-staining ability, various isolates were grown on pine and sweet-gum wood. Discs of sapwood, approximately 2½ inches in diameter and ½ inch thick, were cut from the stems of small living trees and after removal of the bark were immersed for 10 seconds in boiling water, and then placed in sterile Petri dishes. Three discs of each species of wood were inoculated with actively growing mycelium of each of 13 isolates and incubated at approximately 25° C. for two weeks. The isolates used and the results of the inoculations are listed in table 2.

After an incubation period of two weeks, all the inoculated pine discs were completely stained and all the inoculated sweet-gum discs were stained in the outer portions, although the centers were still stain-free. The uninoculated discs of sweet gum and pine remained bright and stain-free, except for chemical brown stain found under the surface of the sweet-gum wood. This chemical stain was distinct in color and distribution from the fungus stain of the inoculated wood. There was no discernible difference in color or extent of stain caused by isolates from stained wood and other sources.

DECAY OF CITRUS FRUITS

Diplodia natalensis has been reported as causing several citrus diseases, including an important fruit decay (6). All the isolates from cotton, citrus, tung, and pear and six from wood (W 2, 3, 4, 5, 6, and 7) were tested for their ability to decay citrus fruit.

Oranges, 2½ to 3 inches in diameter and firm, were secured on the open market. The stem abscission scars were lightly flamed with alcohol and punctured with a sterile scalpel. A small block of mycelium bearing agar was placed over the scars, and the fruits were placed in an incubator at approximately 25° C. Two oranges were inoculated with each isolate and 6 with sterile agar as controls.

Five to 8 days after inoculation most of the oranges were soft and watery from decomposition of the white inner portions and the membranes between segments. The pathogen was reisolated from the center of each orange inoculated with isolates P, T, C 1, C 2, C 4, C 5, W 2, W 4, and W 5, and from 1 of 2 oranges inoculated with each of W 3, W 7, and Cit. The second orange of these latter pairs, both inoculated with C 3, and all controls, showed no decomposition and remained sterile. Since the inoculum blocks were not protected from drying, it seems probable that the few inoculations not resulting in infection were due largely to rapid drying of the inoculum blocks. In another series of inoculations with the isolates that had given some or no infection, the inoculated fruits were placed in closed glass containers for 8 hours prior to placing in the larger incubator previously used. All of these inoculations gave positive results (Table 2).

PATHOGENICITY ON COTTON BOLLS

Diplodia gossypina is one of the fungi causing boll rot of cotton (3). As was pointed out in the introduction, this fungus is considered identical with *D. natalensis*.

Bolls one inch or more in length and growing on plants in the open were inoculated on several dates between June 15 and July 15, 1941. Inoculation was accomplished by cutting a small incision through the wall of the boll, inserting a small piece of mycelium grown on malt agar, and pressing the wound together. In the controls the incision was made but no inoculum was introduced. The isolates used, the number of bolls inoculated, and the number of bolls showing decomposition are given in table 2.

Most of the inoculated bolls exhibited some decomposition in 2 to 5 days. Usually the entire boll turned brown to black on the outside, and the interior was disintegrating rapidly 4 to 5 days after inoculation. Isolates W 8, P, T, and Cit, however, caused little discoloration or decomposition of the boll wall when inoculated into larger bolls, even when decomposition of the interior was advanced. Four to 5 days after inoculation the pathogen was reisolated from the interior of each inoculated boll, except from 1 boll inoculated with Cit and 1 with T. In these cases very little decomposition occurred and only bacteria were reisolated. Although each isolate used caused almost complete disintegration of at least one inoculated boll, the isolates from cotton and isolates W 1 and W 2 distinctly caused more rapid decomposition than did the rest. None of the controls developed black-boll rot symptoms, although a slight browning and

TABLE 2.—*Results obtained by inoculating sapwood discs, orange fruits, stubs and shoots of tung seedlings, and cotton bolls with various isolates of Diplodia natalensis*

Isolate	Ratio: Number of infections/Number of inoculations[a]						
	Stain of sapwood discs		Decomposition of orange fruits		Cankers on tung seedlings[b]		Cotton boll rot
	Sweet gum	Pine	Test 1	Test 2	Stub inoculations	New-shoot inoculations	
C 1	3/3	3/3	2/2	....	0/2	0/3	5/5
C 2	3/3	3/3	2/2	....	0/4	0/3	6/6
C 3	3/3	3/3	0/2	3/3[a]	....	....	6/6
C 4	3/3	3/3	2/2	....	....	....	....
C 5	3/3	3/3	2/2	....	0/3	0/3	....
Cit	3/3	3/3	1/2	2/2	0/5	0/4	7/8
P	3/3	3/3	2/2	....	0/6	0/6	5/5
T	3/3	3/3	2/2	....	0/5	0/3	6/7
W 1	3/3	3/3	....	....	0/4	0/3	5/5
W 2	3/3	3/3	2/2	....	....	....	5/5
W 3	....	....	1/2	2/2	0/3	0/4	5/5
W 4	....	....	2/2	....	....	....	....
W 5	3/3	3/3	2/2	....	1/4	0/4	5/5
W 6	3/3	3/3	2/2	2/2	....	....	....
W 7	3/3	3/3	1/2	2/2	....	....	....
W 8	....	....	....	....	0/4	0/3	5/5
Checks	0/8[c]	0/6	0/6	0/2	0/4	0/5	0/12

[a] The numerator indicates the number of infections and the denominator the total number of inoculations.

[b] *D. natalensis* persisted as a saprophyte on the dead stubs beyond the uppermost bud and at points of inoculation on new shoots where temporary cankers were calloused-off.

[c] Chemical brown stain occurred in both inoculated and noninoculated sweet gum discs. This was distinct from the fungus stain in the inoculated discs.

softening occurred at the incision point. Bacteria were isolated from the browned tissue.

PATHOGENICITY ON TUNG TREES

Diplodia natalensis has been reported as a pathogen on tung (4, p. 224; 9), although it is apparently of little importance on this host.

One hundred seedlings,[1] 9 months old and with the tops removed 6 to 8 inches above ground, were transplanted during the dormant season. One-half of these were lightly fertilized with a complete fertilizer (4–8–4). At the start of shoot growth 40 trees were inoculated on the stub above the sprouts by placing mycelium-bearing agar into an incision through the bark. The isolates used and the number of plants inoculated with each are listed in table 2. Sterile agar was applied on 4 as checks. The wounds were wrapped with wax paper, which was removed a week later. After the sprouts were approximately 0.5 inch in diameter, similar inoculations were made into the shoot growth on 36 remaining plants, with sterile agar on 5 as checks.

With the stub inoculations, *Diplodia natalensis* spread down to the region of the sprout, fruiting copiously in the bark, but there was no indication (7 months after inoculation) that it could spread into the sprout or below the sprout in the stub except in one instance in which a weak plant was killed back to the ground line (W 5). A vigorous root sprout was formed 6 weeks after the inoculation had been made. The checks likewise died back to the sprouts, but more slowly than did the inoculated plants.

In the sprout-inoculated series the incision on the 5 checks healed completely in 2 weeks, while black cankers formed at all incisions into which *Diplodia natalensis* had been inoculated. One month after inoculation strong callous growth had formed at the margins of all cankers preventing further spread of the fungus. Five months after inoculation all inoculated shoots were making vigorous growth and the cankers were almost healed over. Apparently the isolates used, including that originally from tung, are but slightly pathogenic on tung shoots.

SUMMARY AND CONCLUSIONS

Certain comparisons were made among isolates of *Diplodia natalensis* secured from stained lumber or logs and the same or similar *Diplodia* species from cotton, citrus, pear, and tung plants.

There was considerable variation among the isolates used in rate of growth at 37° C., spore size, and general cultural appearance. However, in no cultural characteristic studied did the isolates from stained lumber or logs differ materially from isolates from other sources.

All of 13 isolates tested on pine and sweet gum sapwood proved to be vigorous stainers of these species.

The 14 isolates tested on citrus fruit caused rapid decay. All of the 11 isolates tested on cotton caused black boll rot. None of the 10 isolates inoculated into small tung trees was sufficiently pathogenic to cause more than small temporary cankers that soon calloused over.

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[1] Furnished by the U. S. Field Laboratory for Tung Investigations, Bogalusa, La.

From these studies it seems that the *Diplodia natalensis* causing stain in lumber and logs is, at least from a practical point of view, the same fungus as the isolates secured from several plants. Little fruiting of *D. natalensis* has been observed on stained logs and lumber from which this fungus was isolated. Because of the similarities among isolates of this fungus from stained wood and other sources, it seems probable that some of the inoculum inducing wood stain in logs and lumber is derived from spores produced on such plants as cotton.

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THE INFLUENCE OF VITAMIN B_1 ON THE DEVELOPMENT OF CANTALOUPE POWDERY MILDEW

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INTRODUCTION

Within the last 6 years the effect of vitamin B_1 (thiamin hydrochloride) on plants in their entirety and on different plant organs has been studied considerably. Robbins and Kavanagh (9) point out that there have been conflicting reports regarding the vitamin B_1 stimulation of flowering plants, but that many other organisms have been shown to be heterotrophic, autotrophic, or intermediate with regard to their thiamin requirements. Since these investigators have summarized most of the important literature to date, only papers pertinent to the present investigation will be mentioned. Henry[2] found the vitamin B_1 concentration higher in crown-gall tissue than in stem tissue. This increase was evident shortly after inoculation and before macroscopic galls appeared. Stem tips, however, contained more thiamin than did the galls. When plants were grown at temperatures above the maximum for gall development, the vitamin B_1 content was about the same as that in plants grown at temperatures that permitted galls to appear, indicating no deficiency of vitamin B_1 at the higher temperatures. He concluded that this vitamin does not influence crown-gall development more than any necessary food or growth factor.

There seems to be no published information concerning the influence of thiamin on the host-parasite complex. The present paper summarizes results of an investigation on the development of race 2 of cantaloupe powdery mildew (*Erysiphe cichoracearum* DC.) as affected by thiamin. An abstract of preliminary results has already been published (8).

METHODS AND MATERIALS

Two strains of melons were employed in this study, Powdery Mildew Resistant Cantaloupe No. 45, the common commercial melon of the Imperial Valley, resistant to race 1 of the fungus (5) but susceptible to race 2 (6), and strain 28949, a line completely resistant to race 1 but characterized by necrotic spotting when artificially inoculated with race 2. Macroscopically visible mycelium has never been noticed on plants of the latter strain, either in the greenhouse or in the field. Under the conditions of the present experiments strain 28949 seemed somewhat less vigorous than No. 45. Hereafter, these two strains of cantaloupe may be referred to as the susceptible variety (No. 45), and the resistant variety (strain 28949).

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[2] Henry, Berch W. The relation of vitamin B_1 to crown-gall development. Unpub. Ph.D. thesis deposited in library of Univ. of Wis., Madison, Wis. 1941.

The stock culture of *Erysiphe cichoracearum* used in these tests was obtained from No. 45 plants naturally infected in the Imperial Valley and was, therefore, probably composed entirely of race 2. The fungus was maintained on convenient susceptible plants and occasionally was reinoculated on No. 45 to make certain its pathogenicity had not changed. Since stock plants were susceptible to both mildew races, it is possible that race 1 may have been carried along as a contaminant. No differential hosts have been discovered that are susceptible to race 1 but not to race 2; consequently, it is difficult to recover race 1 from a mixture of the two. Variance analyses (10) were performed on all experimental data.

Soil Experiments

Effect of Thiamin on Disease Development. These experiments were carried out in a greenhouse maintained at a night temperature of about 65° F. and a maximum day temperature generally of 80° to 85° F. but occasionally going as high as 90° or 95° F. Cantaloupe seeds were sown directly in 6 in. pots, and, when cotyledons appeared, the plants were thinned to 5 per pot. Two pots receiving the same treatment were placed side by side and replicated 3 times. Applications of thiamin at the rate of 200 cc. per pot in concentrations of 0.00, 0.01, 0.10, 1.00, and 10.00 p.p.m. were made twice weekly, starting immediately after thinning. Thus in one planting there were 150 plants of each variety. The solutions were made up in tap water not more than an hour before they were used. Supplementary irrigation with tap water was given when required. During the 4 to 6 weeks that elapsed from the time seed was planted until disease notes were taken, 5 to 7 applications of thiamin were made.

As soon as the first true leaves of resistant plants unfolded, they were placed in a glass-sash chamber, accommodating 40 6-in. pots. The plants were then inoculated by blowing conidia from heavily infected leaves into the chamber and allowing them to settle. Blowing the spores from the leaves seemed to result in less clumping and a more uniform distribution of the conidia than did transferring by contact or dusting. The plants were inoculated in the morning and removed to the greenhouse the following morning. Repeated tests of a full chamber of susceptible plants proved the method to be satisfactory in obtaining a uniform, severe infection of the upper leaf surfaces. The following disease index was employed for the resistant variety: Necrosis or collapse of 0 to 20 per cent of the first true leaf was rated as 0; 21 to 40 per cent as 1; 41 to 60 per cent as 2; 61 to 80 per cent as 3; 81 to 100 per cent as 4. Except for one experiment the susceptible variety No. 45 was exposed only to greenhouse infection, and colonies were counted just before they fused together to become indistinguishable. This procedure was followed because it was found that artificial inoculation of this variety produced such severe disease effects that it was impossible to make colony counts. Since the disease appeared in maximum degree more quickly on plants of the susceptible variety notes were taken on them sooner than on those of the resistant strain.

Effect of Thiamin on Growth. Immediately after disease notes were taken on the resistant variety, measurements of height and green weight of tops of both lines were made. This was done usually in the morning and always soon after the pots were watered. Measurements were never made when any of the plants showed signs of wilting.

Since it is practically impossible to maintain susceptible plants free from mildew in a greenhouse used for mildew tests, disease-free melons were grown in another greenhouse. The only one available was unheated and had a night temperature of 50° to 60° F. and day temperatures similar to those of the heated house. Except for the plants being free from disease and growing under a slightly different environment, the experiments with mildew-free plants were duplicates of the inoculated series in the heated house. Growth measurements were made at approximately the same stage of plant development as for the inoculated series.

Dish Experiments

Thiamin Added to Sucrose Solution upon which Inoculated Excised Leaves Were Maintained. Mildew-free plants of both the resistant and susceptible cantaloupe varieties were grown in the unheated greenhouse described above. When 2 to 3 leaves were present, the first and second leaves of approximately uniform size were excised, inoculated by brushing dry spores over the surface, and placed in Petri dishes to which 15 cc. of a sucrose solution and various amounts of thiamin were added. The sugar solution was composed of 100 g. of sucrose per liter of distilled water. Thiamin solutions were made up in such concentrations that a maximum of 1 cc. was required per dish to supply amounts of 1, 10, 100, or 1000 gamma. The dishes were incubated at room temperature. When the first macroscopic sign of mycelium appeared, the colonies were measured to the nearest millimeter and measurements were continued every day until 1 leaf in the series was completely covered with mycelium. There were 10 leaves of each cantaloupe variety for each concentration of thiamin and for the control, making 50 leaves for each strain in each experiment. The treatments were randomized and replicated and the resulting data subjected to statistical analysis.

Thiamin Added to Soil Supporting Mildew-free Plants from which Leaves Were Excised, Inoculated, and Maintained on a Sucrose Solution. First and second leaves from mildew-free plants as described in the section on effect of thiamin on growth were excised after growth measurements were taken. They were then inoculated by the brush method and placed in Petri dishes to which had been added 15 cc. of the sucrose solution described above. Colonies were measured as in the case of the previously mentioned dish cultures. These leaves thus received no external source of thiamin after being excised from the thiamin-treated plants.

EXPERIMENTAL RESULTS

Soil Experiments

Effect of Thiamin on Disease Development. The severity on the susceptible variety and on the resistant strain at about the stage when disease notes were taken is shown in figure 1. Table 1 expresses the means of 4 experiments with 30 susceptible and 30 resistant plants for each of the 5 treatments in each experiment. With the susceptible variety soil applications of thiamin solution at concentrations of 0.01 and 1.00 p.p.m. significantly increased mildew growth over the control, but the difference between the 4 amounts of thiamin were not statistically significant. The mildew increase caused by 0.10 p.p.m. is barely under the value required for significance at the 5 per cent level. The numbers of colonies on the treated plants ranged from 1.3 to 1.5 times the control. With the resistant melon strain the results of the thiamin treatments were more striking, the amount of disease being increased 2.1 to 2.8 times for the several concentrations used, and all of these differences were significantly greater than the control. There were, however, no statistically significant differences between concentrations.

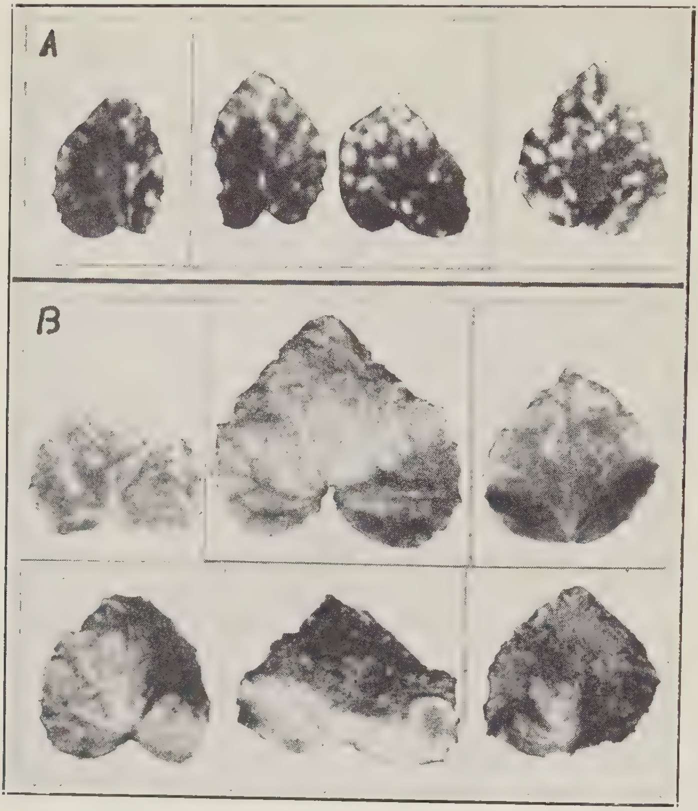

FIG. 1. Powdery mildew on cantaloupe leaves at about the stage when disease notes were taken. A. Leaves of the susceptible variety showing mildew colonies. B. Leaves of the resistant strain illustrating necrosis.

TABLE 1.—*Powdery-mildew development on cantaloupe plants grown in soil to which various concentrations of thiamin solution were added*

Melon variety	Total[a] number of plants	Soil watered with thiamin solution at concentration of					Least signif. mean diff. at 5% level	Least signif. mean diff. at 1% level
		0.00 p.p.m.	0.01 p.p.m.	0.10 p.p.m.	1.00 p.p.m.	10.00 p.p.m.		
Susc.	600	28.98[b]	39.65	38.95	43.55	37.34	9.98	13.11
Res.	600	0.72[c]	1.53	1.58	2.01	1.58	0.77	1.02

[a] Four experiments with 30 susceptible and 30 resistant plants for each of the 5 treatments in each experiment.

[b] Mean number of colonies counted on first true leaf of the susceptible No. 45 variety.

[c] Mean disease index based on condition of the first true leaf of resistant strain No. 28949: 0 = necrosis of up to 20% of the leaf blade; 1 = 21 to 40%; 2 = 41 to 60%; 3 = 61 to 80%; and 4 = 81 to 100% necrosis.

TABLE 2.—*Optimum concentration of thiamin for powdery-mildew development on susceptible and resistant cantaloupe plants in different experiments and different blocks in these experiments, based on averages for each concentration of solution applied to the soil*

Melon variety	Experiment number[a]	Optimum[b] concentration for entire expt.	Block	Optimum[b] concentration for block shown
		p.p.m.		*p.p.m.*
Susc.[c]	I	1.00	A	0.00
			B	1.00
			C	0.01
	II	0.10	A	0.10
			B	0.10
			C	0.01
	III	1.00	A	0.01
			B	0.10
			C	0.01
	IV	1.00	A	1.00
			B	1.00
			C	1.00
Res.[d]	I	1.00	A	0.01
			B	1.00
			C	1.00
	II	0.10	A	1.00
			B	0.00
			C	0.10
	III	1.00	A	1.00
			B	1.00
			C	0.10
	IV	1.00	A	1.00
			B	10.00
			C	10.00

[a] Even though experiments with susceptible and resistant varieties have the same Roman numeral designation, it is not to be construed that they were performed at the same time.

[b] Concentration resulting in greatest average development of powdery mildew.

[c] Cantaloupe No. 45 susceptible to race 2 of the pathogen.

[d] Cantaloupe strain No. 28949 resistant to race 2 of the fungus.

These averages, however, do not tell the whole story. As would be expected, the amount of disease on individual plants varied considerably, especially with the susceptible variety, which was infected by natural means in the greenhouse. This variation extended not only to blocks but to experiments. From table 1 it is apparent that 1.00 p.p.m. of thiamin tended to be the most favorable concentration for mildew development on both the resistant and the susceptible varieties. Table 2 shows the indicated optimum for the different experiments and blocks which contributed the data summarized in table 1. In experiment III with the susceptible melon variety, the optimum concentrations for blocks were 0.01, 0.10, and 0.01 p.p.m., respectively, whereas the optimum concentration for the experiment was 1.00 p.p.m. This apparent discrepancy resulted from the fact that the 0.10 p.p.m. concentration in Blocks A and C and the 0.01 p.p.m. concentration in Block B caused less mildew development than the control, while the 1.00 p.p.m. concentration in Blocks A and B considerably increased the disease over the control, thus bringing the mean of this last concentration for all blocks above that of the other concentrations.

TABLE 3.—*The average green weight and height of tops from diseased and healthy cantaloupe plants grown in soil watered with solutions of thiamin*

Plant condition	Growth measurement	Melon[a] variety	Total no. of plants	Soil watered with thiamin solution at concentration of				
				0.00 p.p.m.	0.01 p.p.m.	0.10 p.p.m.	1.00 p.p.m.	10.00 p.p.m.
Diseased	Weight in g.	Susc.[b]	300	3.32	3.99	3.01	3.46	3.22
		Susc.[c]	150	1.24	1.32	0.98	0.85	0.85
		Res.[c]	450	2.30	1.93	2.40	2.65	2.10
	Height in cm.	Susc.[b]	300	14.16	14.89	13.56	14.10	14.00
		Susc.[c]	150	9.68	9.52	9.36	8.78	8.31
		Res.[c]	450	12.10	10.95	12.35	12.90	11.39
Healthy	Weight in g.	Susc.	450	3.28	2.94	2.92	3.27	3.25
		Res.	450	2.26	2.77	2.19	2.43	2.52
	Height in cm.	Susc.	450	12.06	11.22	11.08	12.06	11.72
		Res.	450	10.01	11.20	9.79	10.80	10.80

[a] Susc. = Cantaloupe No. 45 susceptible to race 2 of the fungus.
Res. = Cantaloupe strain No. 28949 resistant to race 2 of the pathogen.

[b] Exposed only to natural spread of powdery mildew in the greenhouse.

[c] Artificially inoculated with powdery mildew.

The deviation was so great that a few experiments analyzed separately showed no statistically significant stimulation. Nevertheless, when all of the experiments were combined and analyzed, the general effect of treatment was found to be significant. Since these particular experiments were in progress from January to June, it is not surprising that a given type experiment or block in that experiment exhibits different optima when performed at different times of the year under ordinary greenhouse conditions.

Effect of Thiamin on Growth. There was considerable variation in height and weight of plants within treatments, blocks, and experiments. Table 3 shows the means of these values for treatments in all the experiments. While it appears that in most comparisons certain treatments were different from the control, none was significant when the "treatment" variance was compared with "treatment × experiment" variance as error. This was true whether treatment variances were determined as a whole or by single degrees of freedom. Individual experiments also were analyzed statistically, and in all but two there were no significant growth variations. In one experiment with mildew-free plants of the resistant variety, the green weight of plants treated with 0.01 p.p.m. concentration of thiamin was significantly greater than that of similar plants treated with 1.00 or 10.00 p.p.m. concentrations, but none of the thiamin-treated plants differed significantly from the control. In one trial with inoculated plants of the resistant variety, all of the plants treated with thiamin had significantly lower green weight than the control.

On a percentage basis, the growth increases were much smaller than those reported by Bonner and Greene (2, 3), but are not materially different from those reported by other workers (9). Robbins and Kavanagh (9) summarize the situation by saying "It appears that applications of thiamin to intact higher plants is without benefit or the conditions under which it is beneficial are ill-defined."

Dish Experiments

In contrast to the greenhouse trials, there sometimes appeared in the dish cultures a small amount of mycelium on leaves of the resistant strain, but, because of complicating factors, the results on this strain are not included. Areas of discoloration soon appeared in the resistant leaves, but it was difficult to distinguish whether the necrotic spots were caused by powdery mildew, senescence, or contaminating organisms. No necrosis similar to that appearing in the greenhouse was observed.

Thiamin Added to Sucrose Solution upon which Inoculated Leaves Were Maintained. Table 4 gives the results from 3 experiments with leaves of the

TABLE 4.—*Average area of powdery-mildew mycelium parasitizing excised susceptible leaves maintained on a sucrose solution to which various amounts of thiamin were added*

Days from inoculation	Total number of leaves[a]	Quantity of thiamin in 15 cc. of sucrose solution					Least signif. mean diff. at 5% level	Least signif. mean diff. at 1% level
		gamma 0	gamma 1	gamma 10	gamma 100	gamma 1000		
		area in sq. mm.	*area in sq. mm.*	*area in sq. mm.*	*area in sq. mm.*	*area in sq. mm.*		
2	150	0.53	6.37	15.83	1.83	4.23	17.2	23.2
3	150	33.37	59.07	47.53	27.70	50.60	46.5	61.6
4	150	166.4	189.9	155.3	89.4	150.2	126.0	167.0
5	150	459.6	516.3	453.5	267.7	358.0	199.0	264.0

[a] Ten leaves per treatment in each of 3 experiments.

susceptible variety. Although treatments appear to have produced some large differences at 2 days in the means for the total of 3 tests, they are not significant. At 3 and 4 days all lots were quite similar. There was a barely significant difference between 1 and 100 gamma at 5 days, but neither of these values differed significantly from the control or any other concentration. Here again, behavior among experiments was highly variable. In the first test, 2 days after inoculation, 10 gamma produced enormously larger growth than any other treatment by significant odds. In the third test this treatment was exceeded by 1 gamma and in the second test 1 gamma showed no growth at 2 days. Such erratic results indicate the need for more refined and dependable methods for studying the effects of thiamin by this approach. The amount of vitamin B_1 in the leaves at the time they are excised may also contribute considerably to the variability. While it appears that thiamin added to the solution upon which leaves are maintained may stimulate growth of mildew colonies, the evidence is not conclusive.

TABLE 5.—*Average area of powdery-mildew mycelium on excised leaves from susceptible cantaloupe plants grown in soil that had been watered with various concentrations of thiamin solution*

Days from inoculation	Total number of leaves[b]	Soil watered with thiamin solution at concentration of					Least signif. mean diff. at 5% level	Least signif. mean diff. at 1% level
		0.00 p.p.m	0.01 p.p.m	0.10 p.p.m	1.00 p.p.m	10.00 p.p.m		
		area in sq. mm.	*area in sq. mm.*	*area in sq. mm.*	*area in sq. mm.*	*area in sq. mm.*		
3–4[a]	100	3.30	12.30	8.65	9.00	7.20	8.54	11.6
4–5	100	49.5	115.2	55.2	65.3	60.9	78.4	105.0
5–6	100	142.0	308.3	195.4	228.9	161.5	97.8	131.0
6–7	100	340.5	556.0	468.3	453.8	365.1	168.0	226.0

[a] First measurements taken on the day mycelium became macroscopically visible, the incubation period requiring 3 days in some experiments and 4 days in others.
[b] Ten leaves per treatment in each of 2 experiments.

Thiamin Added to Soil Supporting Plants from which Leaves Were Excised, Inoculated, and Maintained on a Sucrose Solution. During 3 to 7 days after inoculation 3 out of the 4 observations showed a significant increase in mildew growth on leaves from plants watered with 0.01 p.p.m. thiamin solution as compared with that on the control plants (Table 5). No other treatment gave results significantly different from the control at any time after inoculation, but the 0.01 p.p.m. treatment resulted in significantly greater mildew growth than some of the other concentrations at 5 to 6 and at 6 to 7 days. It thus appears that a low concentration (0.01 p.p.m.) of thiamin solution tends to stimulate mildew growth under the conditions described. Although only 2 tests were involved, the results may be accepted with some assurance since more elaborate tests with infected plants growing in treated soil have shown that 0.01 p.p.m. significantly increased mildew.

DISCUSSION

Recently a dust containing vitamin B_1 was recommended for the control of cantaloupe powdery mildew in commercial melon fields, the assumption being that the supposedly more vigorous plants resulting from this treatment are resistant to mildew. A preliminary report (8) pointed out that thiamin could not be used effectively for this purpose and that in some cases it actually seemed to stimulate fungus growth. The present work shows that there was a statistically significant increase in parasitic activity due to thiamin, although the conditions under which it occurred were somewhat different from those indicated in the earlier work.

Obligate plant parasites in general seem to develop best when their host is growing most vigorously (13). There is, however, some indication that wheat mildew may be very active, even though the host be not in the best vegetative condition (11). In only one experiment with cantaloupe mildew did thiamin significantly affect growth of parasitized plants. In this case the green weight of thiamin-treated plants of the resistant variety was decreased and mildew was increased by significant odds. The lump analysis of all soil-treatment experiments shows that the growth of *Erysiphe cichoracearum* on the plants was increased significantly, while their height and green weight were unaffected by such use of thiamin. For a longer growth period the greater amount of mildew might have been accompanied by depressed growth of the host.

Since *Erysiphe cichoracearum* has not been cultured in the absence of the living host, the effect of nutrients or hormones on mycelial development are, at present, best studied by indirect methods. Results from an investigation employing such a technique can be accepted only with certain qualifications. Some of the difficulties have been enumerated in connection with studies of another fungus parasite (*Plasmodiophora brassicae* Wor.), which likewise has not been grown *in vitro* (7).

Although thiamin, when supplied to the host plant, tended to promote powdery-mildew development, it is not possible from the present data to be sure that thiamin as such directly stimulated growth of the fungus. Indirect evidence from other lines of work, however, gives some indication that thiamin itself may cause increased parasitic activity. Burkholder and McVeigh (4) found that the vitamin B_1 content of maize leaves varied directly with the nitrogen supply but, inversely, with the phosphorus in the nutrient solution. Turner (12) found that barley and corn, high in nitrogen, were readily attacked by mildew. Wheat inoculated with powdery mildew (*Erysiphe graminis* DC.) and supplied with various molecular proportions of salts was found to be most susuceptible when growing in a solution containing 5 per cent of KH_2PO_4, 47.5 per cent $Ca(NO_3)_2$, and 47.5 per cent $MgSO_4$ and least susceptible in one with 90 per cent of KH_2PO_4, 5 per cent $Ca(NO_3)_2$, and 5 per cent $MgSO_4$ (11). Wingard (13) pointed out that many diseases, particularly those caused by obligate parasites, are increased by high nitrogen and decreased by high phosphorus as nutrients. Thus conditions of mineral nutrition that increase thiamin content have in many cases also increased development of certain fungus diseases. However, light, which also influences the quantity of vitamin B_1 in plants (1), may be a complicating factor in this connection. From the data presented in this paper, we can, therefore, conclude that growth of *Erysiphe cichoracearum* on cantaloupes is either directly or indirectly increased by thiamin.

SUMMARY

Of the two melon strains employed in this study, Powdery Mildew Resistant Cantaloupe No. 45, highly resistant to race 1, was readily attacked by race 2 of *Erysiphe cichoracearum;* while Cantaloupe Strain 28949, after artificial inoculation with race 2, produced necrotic spots, generally without macroscopically visible mycelium. When vitamin B_1 (thiamin hydrochloride solution) was added in various concentrations to soil in which these infected cantaloupe strains were growing, the average number of powdery-mildew colonies on the susceptible variety (No. 45) for the several treatments ranged from 1.3 to 1.5 times that of the control; and the amount of necrosis on the resistant variety (Strain 28949) ranged from 2.1 to 2.8 times that of the control. The results were statistically significant.

When leaves from mildew-free plants of the susceptible variety were excised, inoculated, and maintained on a sucrose solution to which various amounts of thiamin were added, there was no statistically significant effect on mildew development as compared with the control. There was a tendency, however, toward increase of mycelial development with the lower concentrations of thiamin. Thiamin solution applied to soil supporting mildew-free plants of the susceptible variety from which leaves were finally excised, inoculated, and maintained on a sucrose solution, significantly increased mildew growth at 0.01 p.p.m. concentration; but higher concentrations of the watering solution were without consistent significant effect.

Various concentrations of thiamin solution added to soil had no consistent significant effect on the green weight or height of cantaloupe plants growing therein.

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MOLDY CORE OF APPLES IN WISCONSIN

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(Accepted for publication February 14, 1942)

Studies on moldy core of apples have been reported by several investigators. Harrison (4) reviewed most of the literature prior to 1935 and presented the results of comprehensive studies on Gravenstein apples in Nova Scotia. Goss and Doolittle (3), 1915, and MacInnes (5), 1917, reported studies on moldy core. In New Zealand, Brien (1) published recently on the fungi associated with moldy core. Tiller and Cooper (7) investigated the detection of moldy core by X-ray but found it commercially unfeasible. Woodhead (8) found no correlation between pruning practices and the incidence of this disorder. In Argentina moldy core has been studied by Marchionatto (6).

Moldy core occurs annually in susceptible varieties throughout Wisconsin, but it is of minor economic importance. The work reported herein was done at Gays Mills, Wisconsin, using chiefly Dudley and Delicious apples, locally the most susceptible varieties. Dudley, the more important variety in the Gays Mills area, is marketed as a green culinary apple without the complications that might arise from prolonged storage. The losses result from a lowering of the grade of the few fruits visibly affected at the time of picking. Moldy core in Delicious may prove objectionable in advanced cases, since the blackened core cavity is unsightly in a dessert apple. Fortunately, flesh rot is not commonly associated with this disorder in Wisconsin.

MATERIALS AND METHODS

The apples used were of typical conformation and free from complicating defects, such as internal injuries caused by insects or externally visible growth cracks. The samples were picked to represent both the typical exposures and the various size classes of fruit.

The Dudley and Delicious fruits used were usually immature. The samples of Dudley fruit were taken from shortly before harvest until well into the harvest period in 1939 and 1940. In 1941 the samples were picked within a 10-day period before harvest. The Delicious apples were sampled in the latter part of August, a few weeks before their harvest time. When measurements of fruit size were made, the maximum transverse diameter was determined.

To isolate the fungi associated with moldy core and to determine the prevalence of the disorder, a piece of endocarp or a seed was removed aseptically from each fruit and transferred to a Petri dish containing potato-dextrose agar. The plates were incubated for at least 1 week at room temperature. In fruit with no macroscopic symptoms a piece of endocarp was re-

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[1] The writer wishes to thank Dr. G. W. Keitt for helpful criticism and advice during the course of this study.

moved near the distal end of the core, since the fungus enters there. In 1941, moldiness was macroscopically visible in only 62 per cent of the affected fruit. The fungi isolated were identified microscopically, although their gross cultural characters were usually definitive.

EXPERIMENTAL RESULTS AND DISCUSSION

The symptoms of moldy core observed are like those described by Brien (1) and Harrison (4). The endocarp walls and the seeds gradually become overrun and darkened by fungal growth, and in the advanced stages the core cavity is filled with a gray mycelial weft. Occasionally the flesh surrounding the core begins to rot as the fruit approaches maturity. The only reliable color change associated with this disorder in Dudley and Delicious is a precocious development of the yellow ground color, and this occurs only when the infection is at an advanced stage. The flavor of the fruit is unimpaired, unless flesh rot occurs.

Most of the affected fruits had no external symptoms at their respective harvest times. Slight moldiness is not an important defect in fruit for immediate consumption. The growers of the Gays Mills area report no complaints about the development of moldy core into a flesh rot in stored Delicious apples.

The disorder was about equally common in Dudley fruits of all sizes (Fig. 1). Observations indicate a like relationship for Delicious. Harrison (4) found among Gravenstein apples that moldy core was more common in the heavier fruit.

TABLE 1.—*Fungi associated with moldy core in several varieties of apple, Gays Mills, Wisconsin*

Variety of apple	Year	Isolations attempted	Fruits yielding			
			Alternaria sp.	*Fusarium* spp.	*Alternaria* sp. and *Fusarium* spp.	Other fungi
		No.	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Ben Davis	1939	36	27.8	0.0	2.8	2.8
	1940	62	14.5	0.0	0.0	3.2
Delicious	1939	34	79.4	8.8	5.8	2.9
	1940	105	48.5	2.9	8.6	7.6
	1941	150	65.3	7.3	9.3	2.7
Dudley	1939	56	76.8	1.8	10.7	1.8
	1940	301	78.1	0.9	1.9	2.6
	1941	403	71.7	3.7	9.9	8.4
Fameuse	1940	60	6.6	0.0	1.5	1.5
Golden Delicious	1940	29	10.3	0.0	0.0	0.0
McIntosh	1940	51	2.0	2.0	0.0	3.9
Northwestern Greening	1940	51	4.0	2.0	0.0	0.0
Wealthy	1940	51	11.6	0.0	0.0	4.0

It is well known that Delicious apples usually have a calycine sinus connecting the central core chamber with the calyx tube. In the present work this structure was found to exist in Dudley, though less commonly. In its absence in either variety, the distal end of the core cavity was found to be separated from the calyx tube by only a thin layer of tissue. Minute cracks

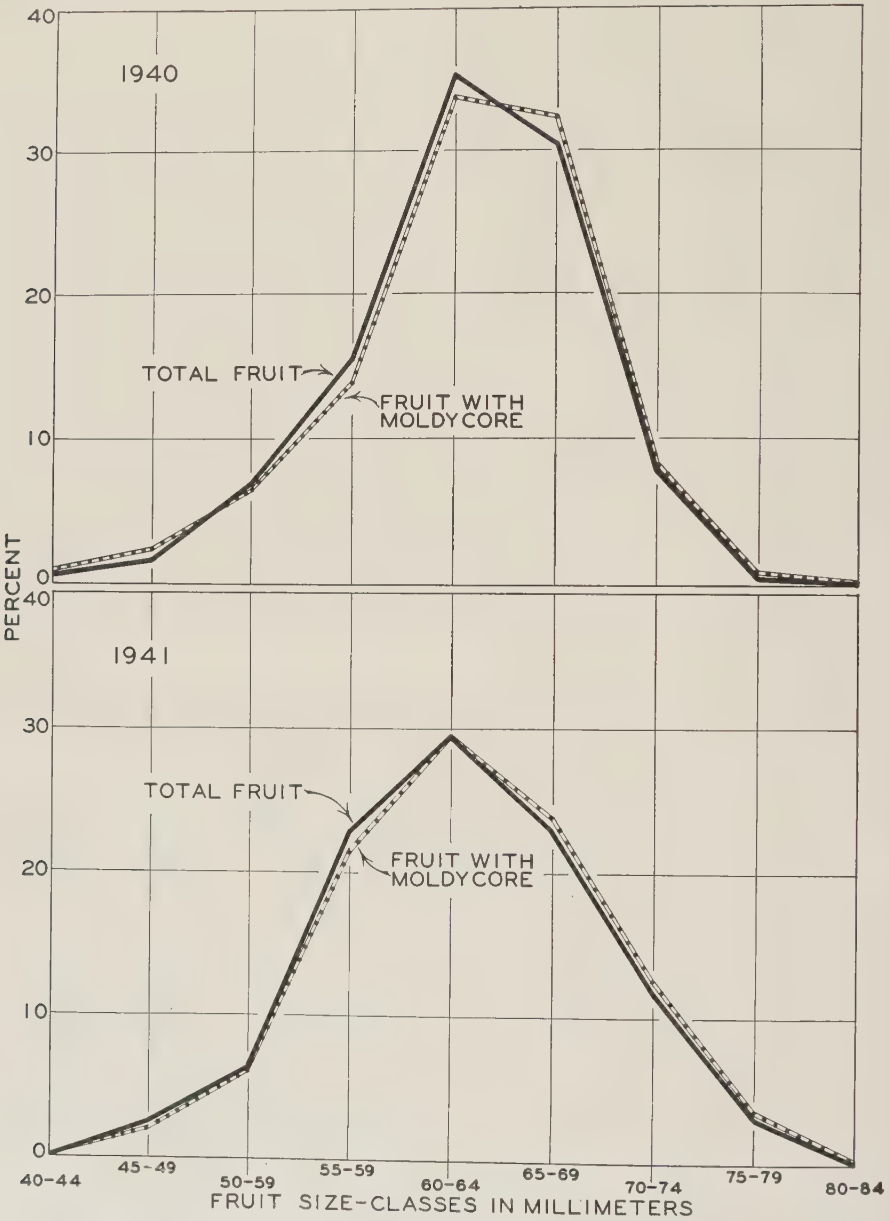

FIG. 1. The incidence of moldy core in relation to the size of Dudley apple fruits. In 1940, 222 of 275 fruits were infected and in 1941, 378 of 402.

may occur in this layer, or the style bases may unite imperfectly, leaving a small opening into the core. In varieties not commonly affected with moldy core, there is no calycine sinus, and usually a few millimeters of apparently solid tissue lie immediately below the calyx tube, effectively barring the entrance of chance invaders.

The fungi associated with moldy core were predominantly of the genus *Alternaria* (Table 1). The isolates of this genus all fell within the limits of the *Alternaria tenuis* group (2). In each season, 1939, 1940, and 1941, the morphological characters of the many isolates were uniform enough to make them members of a single species. Spore size was as variable in monoconidial as in mass isolates. Inasmuch as the *Alternaria tenuis* group includes many named species of uncertain validity, the isolates of *Alternaria* considered in this study will be hereinafter designated as *Alternaria* sp.

Alternaria sp. was occasionally associated with a species of *Fusarium* or with certain other fungi. An isolate of *Fusarium* or of some undetermined genus was obtained alone from a few fruits.

An attempt was made to identify the organisms associated with the flesh rot that occasionally accompanied moldy core. Isolates of both *Alternaria* and *Fusarium* were obtained from the rotted flesh.

Species of *Alternaria*, *Coniothyrium*, *Fusarium*, and *Hormodendrum*, as well as bacteria or yeasts, were isolated in abundance from the floral remnants in the calyx basin. The isolates of *Alternaria* and *Fusarium* were morphologically identical with those from moldy cores. The presence of so many different fungi in the calyx basin, considered with the predominance of *Alternaria* sp. in moldy cores, suggests that the latter fungus may be peculiarly adapted for penetrating into the core and establishing itself (*cf.* 4).

SUMMARY

An account is given of the symptoms of moldy core in the Dudley and Delicious varieties of apple. This disorder occurs in a high proportion of the fruit of these varieties in Wisconsin, but it is of minor economic importance.

Moldy core was equally common in Dudley fruit of all sizes.

Undesirable morphological characters of the fruit predispose Dudley and Delicious apples to infection by the several fungi found in moldy cores.

A species of *Alternaria* of the *Alternaria tenuis* group was the predominant fungus. Species of other genera were found either alone or associated with *Alternaria* sp.

Inasmuch as several species of fungi were found growing in the calyx basin on dead floral remnants, it seems that *Alternaria* sp. may be peculiarly adapted for entering the core and establishing itself.

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A NEW STRAIN OF THE TOMATO LEAF-MOLD FUNGUS (CLADOSPORIUM FULVUM)

L. J. ALEXANDER[1]

(Accepted for publication February 12, 1942)

INTRODUCTION

In the development of the leaf-mold-resistant tomato variety, Globelle, Alexander (2) crossed individuals of the resistant currant tomato, *Lycopersicon pimpinellifolium* Mill. with individuals of the domestic species, *L. esculentum* Mill. varieties Globe and Marhio. During the second crop after the introduction of the variety Globelle, leaf-mold lesions were observed on this variety, and subsequently it was attacked where grown throughout northern Ohio. Observations indicated that a new strain of the fungus had appeared.

Guba (6) studied the resistance of the tomato to Cladosporium leaf mold and came to the conclusion that the existing confusion regarding the partial resistance of certain varieties of the domestic species was due to variability in the growing conditions rather than to the existence of different strains of the fungus. Langford (8), in a study of the parasitism of *Cladosporium fulvum* Cooke, identified 4 physiologic strains of the fungus. However, both Guba and Langford, and others (1, 2, 4 and 9), reported that *Lycopersicon pimpinellifolium* was highly resistant to or immune from all collections of the fungus tested. Also, in private correspondence, D. H. Wenholz of New South Wales reported *L. pimpinellifolium* highly resistant to *C. fulvum.*

Alexander (3) reported the occurrence of a new strain of the fungus capable of attacking Globelle, as well as the collection of *Lycopersicon pimpinellifolium* previously found resistant or immune. This paper is an elaboration of the earlier article and presents additional evidence to show that *Cladosporium fulvum* is not limited in its host range to varieties of the domestic species.

METHODS AND MATERIALS

In a previous article, the writer (1) described the methods of inoculation and disease recording. In the present investigation, the same methods were followed, except that diseased leaf materials, used as a source of spores for inoculation, was collected from different sources. Diseased leaves of Globelle were collected from the glasshouse where the disease first appeared on this variety. Diseased leaves from the susceptible variety Globe were collected from glasshouses in southern Ohio, where Globelle was not adapted and, consequently, had not been grown. In this paper, the spores collected from Globe will be referred to as the "Globe strain" and those collected from Globelle will be referred to as the "Globelle strain."

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[1] Published with the approval of the Director of the Ohio Agricultural Experiment Station.

EXPERIMENTAL

In cross-inoculation tests, Globe and 3 other standard varieties were used as susceptibles. The resistant varieties were Red Currant, Globelle, Bay State, and Veto-mold. The two last-named varieties were introduced as leaf mold-resistant by Guba (7) and Langford, respectively.

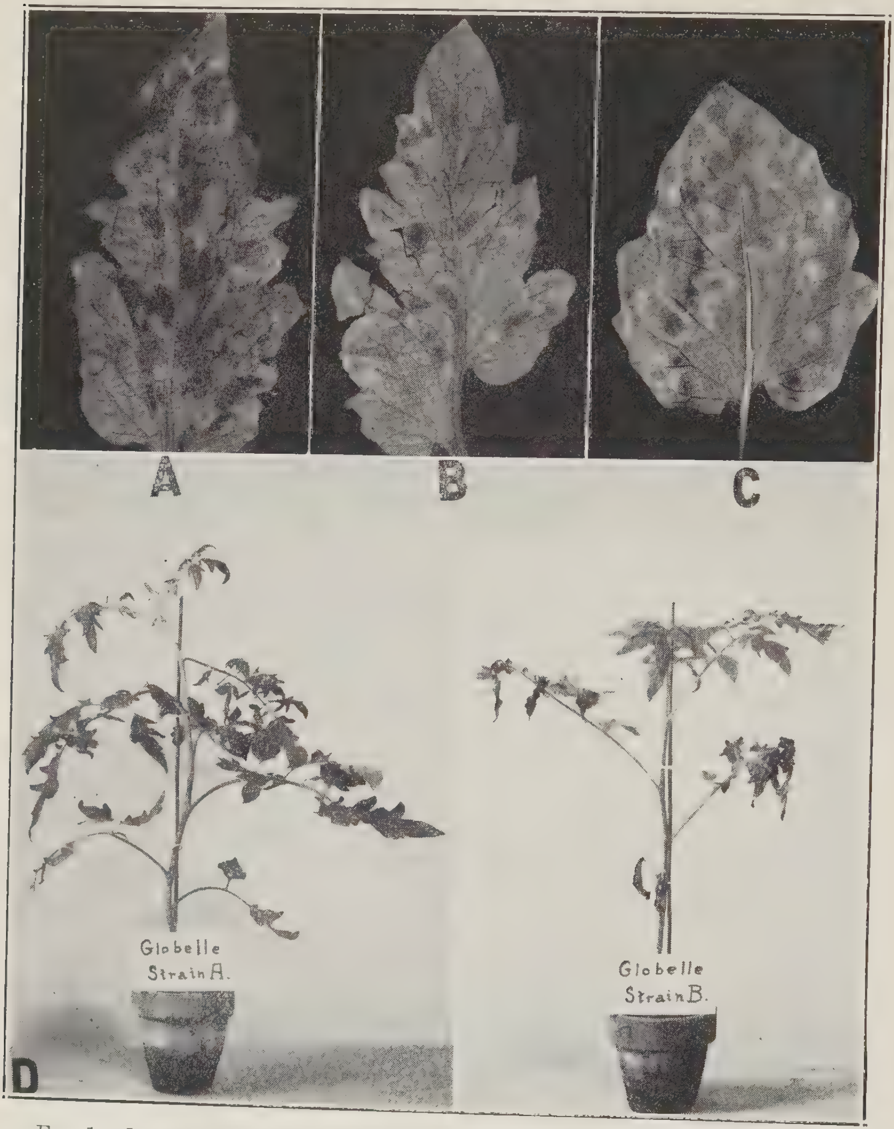

FIG. 1. Leaflets of Globe (A), Globelle (B), and Red Currant (C) naturally affected with leaf mold. D. Plants of the variety Globelle artificially infected with the Globe (left) and Globelle (right) strains of the leaf-mold fungus.

The results of 4 separate cross-inoculation experiments are combined and summarized in table 1.

All plants of the standard varieties were susceptible alike to both strains of the fungus. However, Red Currant and the three resistant varieties were highly resistant to or immune from the "Globe strain," but were susceptible to the "Globelle strain" of the fungus. This difference is illustrated in figure 1, D. In the course of additional work to improve Globelle, these findings with respect to Globe and Globelle have been confirmed. As a further test of the resistance of Red Currant, a few plants were grown along with Globelle in a commercial glasshouse. When the disease appeared on Globelle, it also appeared on Red Currant. A photograph of a naturally infected leaflet of Red Currant is shown in figure 1, C.

Since its introduction, Globelle has been grown in many glasshouses, hence there has been an opportunity to make comparative observations on the severity of the disease on the varieties Globe and Globelle. These observations indicate that Globelle is injured less severely than Globe. Lesions develop more slowly on the one than on the other; consequently, it takes longer for leaves to be killed. This difference is illustrated in figure 1, A and B. The light-colored areas on the leaflet of Globelle are leaf-mold lesions that are developing slowly and have not yet fruited.

TABLE 1.—*Summation of four experiments to demonstrate the existence of two strains of Cladosporium fulvum*

Varieties tested	Globe strain		Globelle strain	
	No. plants resistant	No. plants susceptible	No. plants resistant	No. plants susceptible
Globe	0	61	0	81
Bonny Best	0	8	0	8
Potentate	0	20	0	20
Stirling Castle	0	27	0	26
Red Currant (*L. pimpinellifolium*)	30	0	0	30
Globelle	82	0	0	95
Bay State	16	0	0	15
Veto-mold	31	0	0	16

In a cursory microscopic examination of spores and fruiting structures of both strains of the fungus, no significant differences were noted. Measurements of the unicellular spores of the "Globelle strain" of the fungus averaged $3.89 \times 1.80\ \mu$; spores of the "Globe strain" averaged $3.84 \times 1.74\ \mu$. This slight difference is probably not significant.

DISCUSSION

The origin of the new strain of the fungus is uncertain. The organism is interesting because it was first observed in a commercial glasshouse where many parental lines of Globelle had been grown without showing any evidence of leaf mold. This observation in connection with those of other investigators (4, 6, 8, and 9) who had not observed any evidence of the existence of strains of the fungus capable of attacking the currant tomato indicates that the new strain of the fungus was not previously present.

These observations suggest the possibility that the new strain arose by mutation.

The first observed lesions of the new strain of the fungus were few in number and not typical of leaf mold, but a microscopic examination revealed the presence of typical *Cladosporium* spores and conidiophores. The succeeding crop of Globelle became generally infected. These lesions were typical of leaf mold and some defoliation occurred. Subsequent to the first appearance of the new strain of the fungus it soon appeared in glasshouses throughout northern Ohio and, also, reports of its existence have come to the author either by letter or by conversation with plant pathologists or growers from the greater part of the northeastern United States and once from Texas.

If the new strain of the fungus arose, as suspected, by mutation, it seems possible that seed transmission could account for its rapid dissemination. Opportunities for seed infection were present because much of the early seed of "Globelle" was sold by the grower in whose glasshouse the new strain first appeared. This seed was saved from ripe to over-ripe fruits, picked from vines infected with the Globelle strain. The pulp was extracted and allowed to ferment in the packing shed and finally put to dry on screens in a small plant house. In this connection, it might also be mentioned that Gardner (5) demonstrated that seed transmission can occur when fruits are severely damaged by the leaf-mold fungus.

SUMMARY

The germplasm of the imperfect fungus, *Cladosporium fulvum*, is shown to be sufficiently plastic to permit the evolution of new physiological strains.

The fungus, *C. fulvum*, is not limited in its parasitism to the one host species, *Lycopersicon esculentum*.

The variety Globelle, introduced as resistant to leaf mold, proved susceptible to the new strain of the fungus but less susceptible than Globe.

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DEFOLIATION OF AMERICAN HOLLY CUTTINGS BY RHIZOCTONIA

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(Accepted for publication February 24, 1942)

INTRODUCTION

A disease of American holly (*Ilex opaca* Ait.) cuttings was observed in 1936 in propagation experiments of Neil W. Stuart and Paul C. Marth[1] at this station. In the following year these cutting beds were emptied and refilled with clean sand, but the disease again caused heavy loss. It was noted that the disease first appeared near a wooden partition and rapidly spread to the rest of the cutting bed.

Holly cuttings rooted best when set deep, with the petioles and often part of the leaf blades covered with sand. This deep setting also favored development of the pathogen in question, a species of Rhizoctonia. Because holly cuttings require a month or more to root and the favorable period for propagation may be passed before the disease becomes manifest, it is difficult to replace losses from this disease by starting other cuttings later.

SYMPTOMS

The first symptom of the disease is a cobweb-like effect (Figs. 1 and 2) resulting from adherence of fungous filaments and grains of sand to the under side of leaves that touch the sand. Defoliation may begin in 2 or 3 weeks after the cuttings are set (Figs. 1 and 2). Not long after defoliation black sclerotia may develop on the dead leaves lying on the sand. Sclerotia do not always form on leaves in the cutting bed, but they usually form very readily on leaves of cuttings placed in a glass moist chamber. Another symptom that is sometimes, but not always, present is a zonate leaf spot. It developed on naturally infected cuttings and on cuttings in beds inoculated with a pure culture of the fungus (Fig. 1), but not in uninoculated beds. Isolations from such zonate spots yielded a Rhizoctonia that produced characteristic defoliation symptoms when used in inoculations. This evidence justifies the conclusion that under certain conditions this pathogen which is identical with the species of Rhizoctonia used in other inoculation experiments, may produce a zonate leaf spot. Stahel[2] reported a species of *Corticium* causing a zonate or areolate leaf spot on citrus in Surinam.

PATHOGENICITY

A species of Rhizoctonia consistently isolated from diseased cuttings has proved to be the cause of this disease. On December 11, 1937, sterilized sand was placed in glass moist chambers and mixed with inoculated oats; one dish was left uninoculated to serve as a check. Cuttings were then set in these moist chambers as in a cutting bed. Ten days after setting, the cuttings in the inoculated sand had dropped part of their leaves and nearly all were about to drop. Fungus filaments with enmeshed grains of sand adhering to the under side of the leaves presented the cobweb effect characteristic of the disease in a cutting bed (Fig. 1). Blackening of petioles and of parts of leaf blades was also apparent. The symptoms experimentally produced on holly cuttings in a moist chamber by a pure culture of the Rhizoctonia were therefore similar to those in naturally infected holly cuttings from which the Rhizoctonia was isolated. The reisolated fungus was identical with the inoculant. Cuttings in the moist chamber containing uninoculated sand were alive and showed no evidence of the disease at the conclusion of the test. Later experiments with cuttings set in inoculated and in disease-free beds (Fig. 2) confirmed this evidence obtained from moist chambers.

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[1] Stuart, Neil W., and Paul C. Marth. Composition and rooting of American holly cuttings as affected by treatment with indole butyric acid. Proc. Amer. Soc. Hort. Sci. **35**: 839–844 (1937). 1938.

[2] Stahel, Gerold. *Corticium areolatum*, the cause of the areolate leaf spot of citrus. Phytopath. **30**: 119–130. 1940.

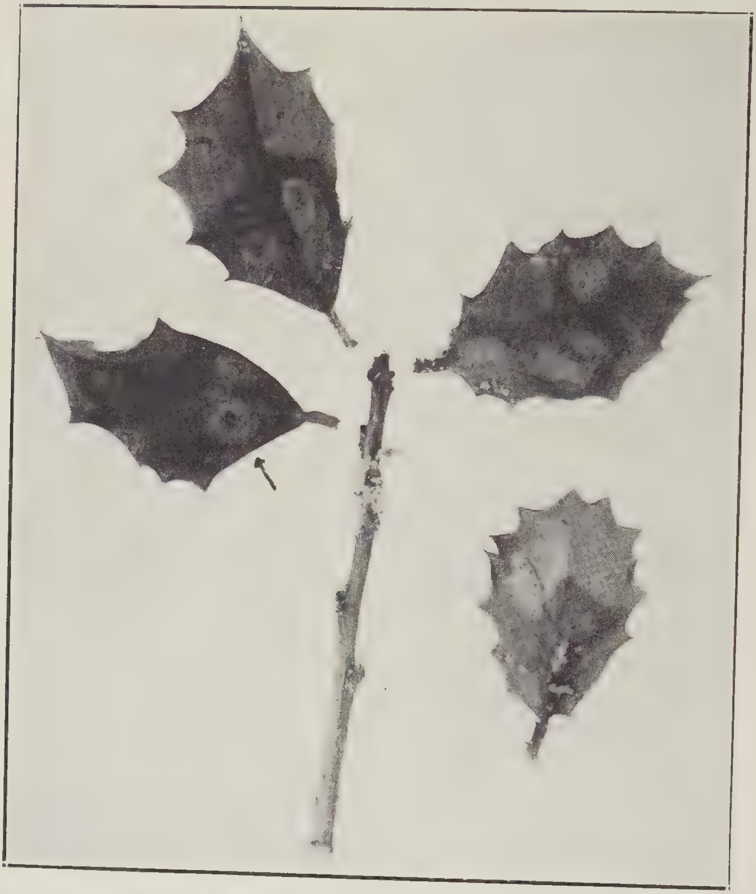

FIG. 1. Diseased holly cutting from an inoculated cutting bed, showing the advance of the disease up the petioles, the adherence of sand to the affected parts, and the zonate leaf spot (indicated by arrow).

Cultures of this fungus were determined to be *Rhizoctonia solani* Kühn,

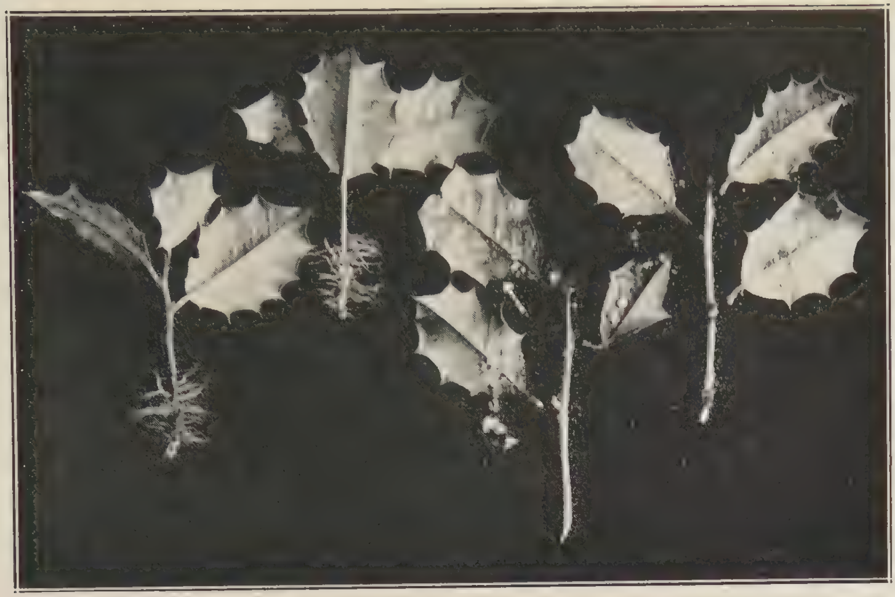

FIG. 2. Two healthy holly cuttings from uninoculated cutting bed (left). Two diseased cuttings from inoculated bed; a zonate leaf spot is indicated by the arrow (right).

by William Davis and by Ernest Wright of the Division of Forest Pathology, U. S. Bureau of Plant Industry. Comparison of this strain with a species of *Rhizoctonia* isolated from potato showed only minor differences in color and character of growth. This fungus proved to have a very narrow temperature range for good growth in Petri plates of potato-dextrose agar, as recorded in Table 1. Growth was extensive at temperatures near 25° C., but very restricted above 30° or below 15° in the 48-hour period. At 20° C. there was considerable elongation of hyphae but the hyphal mat was very thin, and at 15° it was decidedly subnormal in appearance. In the presence of sufficient moisture the development of this disease may be dependent upon suitable temperature. Holly cuttings rooted favorably at a temperature near the optimum for growth of this fungus, but the effect of temperature on rooting was not a subject of experimentation.

TABLE 1.—*Effect of temperature on growth of Rhizoctonia from holly on potato-dextrose agar*

Temperature	Diameter of hyphal mat after 48 hours	Character of hyphal mat
° C.	*Mm.*	
5	0	....
10	0	....
15	16.8	Very thin
20	41.5	Thin
25	73.5	Very dense
30	55.6	Thin
35	0	....
40	0	....

Cuttings of a number of plants usually propagated by cuttings were set in a bed infested with the holly Rhizoctonia and also in an uninoculated bed. This test included begonia (*Begonia* sp., Lorraine type), buddleia (*Buddleia davidi* Frauch), carnation (*Dianthus caryophyllus* L.), chrysanthemum (*Chrysanthemum* sp.), fuchsia (*Fuchsia* sp.), geranium (*Pelargonium hortorum* Hort.), heliotrope (*Heliotropium* sp.), lantana (*Lantana camara* L.), peristrophe (*Peristrophe angustifolia* Nees.), and snapdragon (*Antirrhinum majus* L.). All the plants tested showed some degree of infection and gave much poorer rooting in the inoculated than in the noninoculated bed. In the inoculated bed cuttings of highly susceptible plants, such as begonia, died in a short time while more resistant ones, such as the chrysanthemum, showed moderate injury with survival of some of the cuttings. These results indicate that the holly Rhizoctonia may kill the cuttings or adversely affect the rooting of a wide variety of plants.

SOURCE OF INFECTION

When cuttings from different holly trees and from different positions on such trees, including limbs touching the ground, were set in sterilized sand in moist chambers no disease developed. Further evidence that holly cuttings are not the usual source of infection was supplied by other experiments reported in this paper in which holly cuttings taken from the open and set in a clean cutting bed remained disease-free throughout the rooting period. Although this evidence does not preclude the possibility that holly cuttings may sometimes carry the fungus, it is probable that infection usually originates from contaminated greenhouse material.

CONTROL EXPERIMENTS

In testing chemical treatments the cutting bed was divided into two comparable parts; one was inoculated with the pathogen, and the other was kept disease-free, as a check on the effect of the chemical or other treatments on disease-free cuttings. The fungus, cultured on either steamed oats or Tochinai's[3] solution, was allowed to grow in the inoculated portion of the cutting bed for 2 to 3 days before the chemicals were applied either in solution or in suspension. The propagating case was then kept closed for 12 or more days before the cuttings were set, and again after they were set. The bed was supplied with electrical bottom heat thermostatically controlled at 24° to 27° C. When most of the cuttings were well rooted the bed was cleared and the results recorded. Such experiments were carried on in four successive years.

None of the chemicals tested[4] afforded control of the disease under the conditions of the tests; and all treatments reduced the proportion of rooted cuttings in the uninoculated bed. Applications of disinfectants to cuttings after they were set resulted in chemical injury without control of the disease.

An important result of these experiments was the absence of diseased

[3] Tochinai, Y. Comparative studies on physiology of *Fusarium lini* and *Colletotrichum lini*. Jour. College of Agr. Hok. Kardo Imp. Univ. **14**: 171–236. 1936.

[4] Acetic, sulphuric, hydrochloric, a-naphthyl acetic, and phosphoric acids, sulphur, ferrous sulphate, potassium permanganate, cuprous oxide, aluminum sulphate, ammonium sulphate, calcium superphosphate, Bordeaux mixture, mercuric chloride, at various concentrations.

plants in the noninoculated bed, which was separated from the heavily infected bed by a 6-inch board only. In preparation for the experiment, the bed had been cleaned of sand, disinfected with 1 : 50 formaldehyde solution, and refilled with clean fresh sand. Care was exercised to prevent the carrying of the fungus from the diseased to the noninoculated bed.

Throughout 4 years shallow setting of cuttings also was tested as an accessory control measure. Cuttings were set in a contaminated and also in an uninoculated cutting bed; half of the cuttings in each bed were set shallow so that no leaves were covered with sand, the rest were set deep, so that the petioles and part of the leaf blades were covered with sand. Usually, after about a week, the deep-set cuttings in the contaminated bed showed fungus growth and some leaf shedding, while the shallow-set cuttings showed no evidence of leaf shedding. After 2 weeks the deep-set cuttings showed considerable rhizoctonia infection and defoliation, while the shallow ones showed infection and defoliation only where they were set too close and the leaves touched each other or the sand. In the noninoculated beds both deep-set and shallow-set cuttings consistently remained healthy. Results do not justify recommending shallow setting as a sole perventive measure.

Since *Rhizoctonia solani* does not readily sporulate, dissemination by spores is not common. The fungus, however, does produce an abundance of hyphal growth, which is readily disseminated as mycelial strands and fragments that adhere to various objects. Although the disease is thus readily spread, it has been possible to maintain an experimental disease-free bed adjacent to a heavily diseased one. These experiments show that sanitation affords practical control. If the disease has been present in a bed, the sand should be removed and the bench washed with a disinfectant, such as 2 per cent formaldehyde or 2 per cent copper sulphate solution. The bed should be refilled with fresh clean sand or sterilized sand, and every effort should be made to prevent recontamination. Since *Rhizoctonia* may live on plant debris in the soil, it seems best to avoid taking cuttings from holly branches that touch the ground.

SUMMARY

A leaf-drop disease of American holly cuttings, caused by *Rhizoctonia solani*, is described. The pathogenicity of the fungus was demonstrated by the usual means. The fungus in culture showed a restricted temperature range with the optimum between 25° and 30° C. No positive evidence was obtained that the disease is harbored on holly plants from which cuttings were made. When heavily contaminated cutting beds were treated with a number of disinfectants and cuttings were set after a lapse of 12 or more days none of the disinfectants gave satisfactory control. However, thorough sanitary measures gave effective control. These included renewal of the cutting bed with fresh clean sand after washing the bench with a disinfectant, and the taking of precautions against recontamination.

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RELATIVE CONCENTRATIONS OF TWO STRAINS OF CURLY-TOP VIRUS IN TISSUES OF SUSCEPTIBLE AND RESISTANT BEANS

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(Accepted for publication February 17, 1942)

While investigating the relationship of curly-top virus to root tips of sugar beets it was sometimes difficult to obtain sufficiently large root tips from diseased beets. This difficulty directed attention to beans (*Phaseolus vulgaris* L.), which, in the early stages of curly top, produce somewhat larger root tips. Therefore, in some of this work bean root tips were studied along with root tips of sugar beets.

This paper is an account of experimental results on factors affecting virus concentration in beans. Two strains of curly-top virus served as inoculum for 2 varieties of beans differing in resistance to curly top.

Curly-top virus strains 1 and 4 were chosen from those described by Giddings.[1] The bean varieties were Great Northern U.I. 81, a selection made by Pierce[2] and reported somewhat resistant to curly top by Murphy,[3] and Bountiful, an extremely susceptible commercial variety. Under greenhouse conditions and when inoculated on young leaves, U.I. 81 continues to grow, although obviously affected by virus strain 1. With Bountiful, however, Giddings[1] showed that infection by virus strain 1 caused a loss of leaves, complete cessation of growth, and early death.

Plants of each variety were inoculated with curly-top virus strain 1 and later the root tips and shoot tips were tested for virus concentration, the former about 1 week after inoculation. In each experiment 20 root tips (5 mm. length) of each variety were cut and macerated separately in a 5 per cent sucrose solution. Non-viruliferous beet leaf hoppers were fed on these preparations and then caged singly on susceptible sugar beet seedlings. The percentages of infection in the parallel series of test plants afforded a comparison of the virus concentrations in the 2 bean varieties. Shoot tips were tested by expressing the juice and feeding it to non-viruliferous leaf hoppers.

Table 1 shows the results of parallel experiments with curly-top strain 1 on the 2 bean varieties. Since the number of test plants varied among the experiments, infections are shown as percentages. These results show that in 19 out of 20 tests more of the beets became infected when inoculated with virus from the susceptible bean variety than when inoculated with virus from the resistant variety. In the 13th test, percentages of infection were equal. Obviously, the virus was regularly more concentrated in the root

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[1] Giddings, N. J. Studies of selected strains of curly top virus. Jour. Agr. Res. [U.S.] 56: 882–894. 1938.

[2] Pierce, W. H. Resistance to common bean mosaic in the Great Northern field bean. Jour. Agr. Res. [U.S.] 49: 183–188. 1934.

[3] Murphy, Donald M. A Great Northern bean resistant to curly top and common bean mosaic viruses. Phytopath. 30: 779–784. 1940.

TABLE 1.—*Infection percentages on test beet plants, indicating comparative concentrations of curly-top virus strain 1 in tips of roots and shoots of (a) susceptible bean variety (Bountiful) and (b) resistant variety (U.I. 81)*

Experiment	Virus source			
	Susceptible variety		Resistant variety	
	Number of sugar beets inoculated	Percentage infected	Number of sugar beets inoculated	Percentage infected
	Extract from root tips used[a]			
1	24	43.5	24	4.2
2	24	50.0	24	0.0
3	24	20.8	24	0.0
4	24	50.0	24	0.0
5	24	25.0	24	0.0
6	13	38.6	15	6.7
7	22	63.6	24	20.8
8	20	55.0	20	5.0
9	24	50.0	24	4.2
10	20	25.0	15	6.3
11	20	35.0	20	5.0
12	20	65.0	20	0.0
13	20	5.0	20	5.0
14	20	70.0	24	4.2
15	20	35.0	20	5.0
	Expressed juice from shoot tips used			
16	20	50.0	20	0.0
17	20	10.0	20	0.0
18	20	30.0	20	10.0
19	16	43.8	20	10.0
20	20	25.0	20	0.0

[a] 20 root tips (5 mm. long) of each variety were macerated in 5 per cent sucrose solution.

tips of the susceptible variety. Limited evidence indicates the same is true for shoot tips.

In experiments by Giddings[4] and in some of the tests included in table 1

TABLE 2.—*Infection percentages on test beet plants, indicating comparative concentrations of virus in root tips of Bountiful beans infected with (a) curly-top virus strain 1 and with (b) curly-top virus strain 4*

Experiment	Curly-top virus strain 1		Curly-top virus strain 4	
	Number of sugar beets inoculated	Percentage infected	Number of sugar beets inoculated	Percentage infected
1	24	50.0	24	12.5
2	32	34.4	14	14.3
3	24	45.8	24	0.0
4	24	37.5	24	16.7
5	22	63.7	22	27.3
6	20	60.0	24	0.0
7	15	26.7	12	25.0
8	20	70.0	20	30.0
9	20	35.0	20	10.0

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[4] Giddings, N. J. Unpublished data.

it was observed that a small amount of the virus was usually obtained from U.I. 81 plants soon after symptoms were detectable, but very rarely in later periods of growth of the infected plants. Apparently, shortly after infection occurs some factor in this resistant variety inactivates the virus or inhibits its further multiplication.

Further studies were conducted in which virus strain 1 and the less virulent strain 4 were used to inoculate the susceptible variety Bountiful. Tests of virus concentration in the root tips were conducted as before. These results reveal that in all cases infection percentages were greater when inoculations were made with virus from beans infected with strain 1 (Table 2). The differences were large in 8 of the 9 tests made. Evidently the concentration of the virulent strain was consistently higher than that of the less virulent strain.

These tests demonstrate that in the resistant bean, U.I. 81, virus strain 1 is promptly inactivated or multiplication is quickly inhibited. In highly resistant sugar beets, on the contrary, Giddings[1] has found strain 1 to persist. The tests also show that in the root tips of the susceptible bean, Bountiful, the virulent strain 1 promptly reaches a higher concentration than does the less virulent strain 4. This behavior is in contrast to that found in root tips of susceptible beet varieties[5] in which strain 4 reaches a slightly higher concentration than does strain 1.

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[5] Lackey, C. F. Unpublished data.

PHYTOPATHOLOGICAL NOTE

Susceptibility of Solanum species to Fusarium solani var. *eumartii.*—The lack of information concerning the occurrence of fusarium wilt in wild tuber-bearing species of *Solanum*, and the possibility of discovering resistant parent material for breeding purposes, led the writers to test a number of species kindly supplied by Donald Reddick. The tests were made by growing the seedlings in inoculated, sterilized and unsterilized soil, as described by the writers.[1]

Typical foliage and tuber symptoms of the disease were produced in the seedlings, and the pathogen was recovered in pure culture. On the basis of these tests the following species proved susceptible to the disease: *Solanum acaule, S. demissum* (Reddick 178, 418, and 519), *S. chacoense, S. commersonii* ("Blanca" and "Colorado," from Las Delicias, Argentina), *S.* sp. from Chile, P. I. 129381, *S. antiporiczii.*—R. W. Goss and J. H. Jensen, Nebraska Agricultural Experiment Station, Lincoln, Nebraska.

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[1] Jensen, J. H. and R. W. Goss. Infection of first-year potato seedlings with *Fusarium soloni* var. *eumartii.* Amer. Potato Jour. **18**: 239–242. 1941.

BOOK REVIEWS

HAYES, HERBERT KENDALL, and FORREST RHINEHART IMMER. *Methods of Plant Breeding.* (1st. ed. 432 pp., 37 figs. McGraw-Hill Book Co., Inc., (New York and London). 1942. $4.00.

A textbook designed primarily for the use of students and workers interested in field-crop improvement through plant breeding. Agronomists, phytopathologists, economic botanists, and other allied professional groups also should find this text especially valuable as a reference. The senior author, who has spent his entire career specializing in the teaching and practice of plant breeding, is unusually well qualified to prepare a work of this kind. Hence, many valuable data are presented and numerous outstanding examples of the best methods of breeding crop plants are discussed. The junior author's contribution to the book on methods of field-plot technique, experimental design, and statistical analysis is equally outstanding, presenting timely and up-to-date information on these subjects. Although still a young man, he has attained an eminent position among authorities in his chosen field of research.

In the preface, the authors have expressed concisely, yet rather completely, in simple language the general nature and use of the material presented in the text. ''The subject matter presented . . . has been used in both undergraduate and graduate courses. . . . The undergraduate course is taught only to junior and senior students. The graduate courses are given for the purpose of teaching standardized methods of breeding for particular categories of breeding problems and to present the current viewpoint when the most desirable method of breeding is not so well known. This is with the belief that each of the various methods of hybridization, including the pedigree method of selecting during the segregating generations, the bulk method with self-pollinated plants, the backcross method, and convergent improvement, has certain advantages and disadvantages that make it desirable under some conditions and less desirable for other breeding problems.''

''Methods of field plot technic, experimental design, and statistical analysis with particular reference to plant breeding problems have been discussed, including some of the newer methods. The necessary statistical tables have been included with the permission of the original publishers.''

The book consists of 22 chapters, and is illustrated with 37 figures comprising halftones, tables, and graphs. The general topics for each chapter are: The role of plant breeding; the genetic and cytogenetic basis of plant breeding; mode of reproduction in relation to breeding methods; techniques in selfing and crossing; the pure-line method of breeding naturally self-pollinated plants; hybridization as a method of improving self-pollinated plants; the backcross method; breeding for disease and insect resistance; inheritance in wheat, oats, barley, and flax; methods of selection for special characters; development of methods of corn breeding; inheritance in maize; controlled pollination methods of breeding cross-pollinated plants; seed production; some commonly used measures of type and variability; field-plot technic; randomized blocks, Latin squares, and χ^2 tests; correlation and regression in relation to plant breeding; and multiple experiments, including methods of testing a large number of varieties and the analysis of data expressed as percentages. These chapter titles briefly set forth the contents of the book, which might be considered sufficient for a review, even without further comment.

The authors have deemed it unwise to attempt a complete review of the present status of the genetics of many crop plants, because such a review soon would become obsolete, so rapid is the advancement in the field of genetics, or more particularly in the field of cytogenetics. Brief reviews of the mode of inheritance of important characters of leading crop plants, such as corn, a typical cross-pollinated plant, and the small grains, such as wheat, oats, and barley, typical close-pollinated plants, have been included to illustrate to the reader and student the value of a knowledge of inheritance as an aid to the planning and development of breeding programs. The present status of corn breeding, the leading cross-pollinated economic crop plant, is reviewed in considerable detail, since many of the studies made with corn and the results obtained therefrom are basic to a complete understanding of principles of breeding, particularly for other cross-pollinated plants.

The authors have emphasized, and rightly so, the newer developments in plant-breeding methods that have contributed much to crop improvement. These include polyploids in relation to plant breeding, colchicine as a polyploidizing agent, linkage groups in barley and corn, convergent improvement in corn, the artificial production of epiphytotics of the rusts and smuts of the small grains, wheat-meal fermentation tests, etc. Special emphasis also is placed on the value of backcrossing and backcrossing-breeding programs to obtain more quickly certain goals in plant breeding.

The chapter on seed production presents valuable information on the first increase of seed of a new variety, seed certification and registration, crop improvement associations, etc.

In the preparation of this new book, it is evident that the former well-known and widely used text "Breeding Crop Plants," by H. K. Hayes and R. J. Garber has been used freely. It, therefore, may be stated in all fairness to the authors that "Methods of Plant Breeding" is "Breeding Crop Plants" revised, brought up-to-date with the addition of much new knowledge, particularly of cytogenetics, methods of field plot technique, experimental designs, and statistical analysis. In short, it is a much more complete book than its predecessor, and undoubtedly will receive wide recognition.

The book is well organized, with the possible exception of the chapter on seed production, which may belong more properly to a text on crop production. It is well written and quite free from errors of diction, spelling, etc., and is very readable. In general, this is the most concise, yet complete, text on the subject which has appeared to date. As a consequence, "Methods of Plant Breeding" should meet with almost universal favor among all classes of students and research workers who are interested in crop improvement through plant breeding.

The book is adequately documented with a list of 401 literature citations. These include the more important contributions by workers to plant genetics, plant breeding, and other closely related subjects, and will be of great value to the student for reference. A glossary of genetic, plant breeding, and taxonomic terms follows the section on literature citations. There is appended a group of tables presenting values and constants employed in statistical analysis. These necessary tables contribute much toward a "rounding out" of the text. They also add much to its usefulness.

If the book is deficient in any respect, it is in the number of illustrations. More illustrations of an appropriate nature would have materially improved the text; especially at this time when pictures and drawings are being used more and more to present cardinal facts in all fields of endeavor. Modern textbooks also call for some colored pictures for illustrating certain pertinent subjects, and add much to their appearance and value.

Unfortunately, a few of the halftones used in the book are quite poor and should be replaced by better ones in the first revision by the authors.—T. R. STANTON, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, Washington, D. C.

REED, HOWARD S. *A Short History of the Plant Sciences.* The Chronica Botanica Company (Waltham, Massachusetts). 1942. 320 pp. $5.00.

This book is designed for the average graduate student in our universities, rather than for the specialist in science or in history. Recognizing the limitations of one individual in treating a subject so broad as the history of the plant sciences, the author has chosen to deal chiefly with subjects with which he felt more familiar, referring the reader to recent works in which other writers have treated certain important fields (systematic botany, phylogeny, paleobotany, genetics, plant breeding, evolution, and recent work on growth, tropisms, and hormones).

The book contains 320 numbered pages and 37 figures. The chapters and the pages devoted to each are as follows: Introduction, 6; gardeners and herbalists of antiquity, 23; the nascent period, 15; the retrogressive period, 12; the renascent period, 24; the seventeenth century, 14; the eighteenth century, 18; gardens and other things, 13; plant geography in the nineteenth century, 9; morphology, 19; cytology, 22; the water economy of plants, 21; the fixation of carbon by plants, 18; the assimilation of nitrogen, 12; the fixation and metabolism of nitrogen, 14; plant nutrition, 13; mineral constituents in metabolism, 12; mycology, 19; plant pathology, 24; significant names in the history of botanical science, 1.

The author observes that perhaps he has spent too much time on early developments. The reviewer regards the eight chapters that trace developments from the dawn of history through the eighteenth century as one of the most valuable parts of the book. Here a very scattered and difficultly accessible literature is given scholarly and well-documented treatment.

A short history of the plant sciences encounters heavier going in the chapters in which the several fields are treated. Out of a great mass of material that has not yet received adequate historical interpretation and evaluation by workers in the fields concerned, contributions are selected and interpreted. On the whole one marvels that a single individual, even with the benefit of counsel from colleagues in several fields, should have covered so much so well. Nevertheless, the reviewer finds many places, especially in the fields with which he is more familiar, in which he would prefer a different selection of material or a different interpretation. In the chapter on mycology, for example, Micheli's epoch-making work receives four lines, without mention of its nature or interpretation of its significance. Yet, Fontana, whose contribution was minor in comparison, is given more than a page. No mention is made of Buller's valuable account of Micheli's work in its historical setting. What was more fundamental to the early development of mycology and the mycological basis for pathology than Micheli's experimental demonstration that fungi reproduce by means of spores and are therefore autonomous organisms, rather than evanescent products of fanciful or superstitious origin?

A difficulty inherent in the plan of the work is encountered in the attempt to trace the development of major lines of research to their current position. It is not surprising that in covering so broad a field and including recent work some minor contributions receive consideration, while others of much more significance do not, and that in some cases the position of the problem has been drasticallly changed by recent work not treated.

An error that should be corrected is the assignment to the career of Winogradsky in the list of 50 significant names in the history of botany the closing date of 1934. Colleagues advise the reviewer that Winogradsky was very much alive in 1938 and, insofar as they are informed, may still be.

The book should very usefully serve the purpose for which its author designed it, a guide to reading in the history of the plant sciences. As such it should be very welcome, not only to the graduate student, but to all who are seriously interested in its field. It should not be mistaken for what it was not intended, a definitive and authoritative history of this broad field. This can be written, if ever, only after developments in the many branches of plant science have been adequately interpreted and evaluated by those intimately familiar with the fields concerned. It is to be hoped that Professor Reed's stimulating work will encourage those who cultivate the several fields of plant science to put their historical houses in order, so that students may be able to see the development of their science in its true perspective and the task may be lightened for future writers who have the courage, energy, and talent to attempt to treat the history of broader fields.—G. W. KEITT, University of Wisconsin, Madison, Wisconsin.

THE WAR EMERGENCY COMMITTEE OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

At the Dallas Meeting of the American Phytopathological Society, after a panel discussion lasting the entire afternoon, action was taken authorizing the appointment of a War Emergency Committee of the Society, the personnel of which was published in the September, 1942, issue of PHYTOPATHOLOGY.

The general objectives formulated by the committee are as follows:

1. Provide for more adaquate plant-disease quarantines, foreign and domestic, to guard against introduction and distribution of new and destructive disease organisms.
2. Intensify plant-disease surveys to detect as soon as possible new plant-disease introductions and show where control efforts should be concentrated.
3. Summarize and codify known control measures, make them easily comprehensible and available to extension men and growers, and encourage more adequate extension work in plant pathology.
4. Attempt to get necessary priorities on chemicals and machinery used in controlling diseases.
5. Concentrate effort on necessary experimentation and research designed to improve the effectiveness and economy of plant-disease control measures, by cultural practices, chemical treatments, and resistant varieties.
6. Summarize, and make available information regarding preservation of food and other products in storage and transit, and provide for necessary studies to meet new situations.
7. Scrutinize present basic and long-time research projects with a view to procuring support for those designed to yield facts and principles on which important procedures are based and those that could not be interrupted without serious loss of materials, accumulated results, and experience.
8. Maintain adequate personnel.

It is desirable and necessary to cooperate to the utmost with the United States Department of Agriculture, the State Colleges of Agriculture and Agricultural Experiment Stations, the National Research Council, industrial organizations, and all other agencies that may be useful in the attainment of the general objectives.

All of the subcommittees are functioning and have agreed to issue brief monthly reports and occasional summaries of pertinent information.

At the Summer Meetings of The American Phytopathological Society, at Toledo, Ohio, June 25 and 26, 1942, considerable time was devoted to a discussion of the work of the War Emergency Committee as a whole, and to reports of the subcommittees. Following is a brief summary of the viewpoints expressed and the actions taken:

It is being recognized increasingly that "Food will win the war and write the peace" as expressed by Secretary of Agriculture, Claude R. Wickard. It is evident that there are now very important problems in protecting crop plants against diseases; that the problems become more acute because of shifts in crop production required by the exigencies of the war, and because of the increased importation of propagative parts of plants from other countries and wider distribution within the United States.

To aid agriculture in its immediate war job, research plant pathologists are crystallizing accumulated research results into simple instructions on how best to check losses from serious plant diseases of important war-time crops, or Victory Gardens. They also are directly assisting extension workers and others in planning effective disease-control programs after studying local conditions and adapting scientific findings to local needs and resources. This involves a type of engineering study, or clinical investigation, essential to successful application, on the farm, of knowledge coming from basic research. Special short-time emergency studies and pilot tests, indispensable to efficient war production, were recognized as suitable for support from appropriate federal-grant funds. The great value of voluntary teamwork shown by the Society's group research activities argues in favor of handling many war-time regional and national plant-disease problems through similar cooperative arrangements.

The following fields of research were cited as among those important to the Nation's war effort: A better organized Nation-wide plant disease survey service, fostered by the survey subcommittee, for effective direction of crop protection programs; work on new and improved fungicides and crop-protection methods led by the fungicide subcommittee; development of disease-resistant crop varieties with coordinated local tests under special subcommittees; research led by the seed-certification and seed-treatment committees on problems basic to certification or treatment of seed and planting stocks to reduce losses from seed- and plant-borne diseases; research on rotations, chemical treatment, and cultural management of soils to reduce losses from soil-borne diseases; coordinated research on virus diseases of plants with entomologists helping on insect carriers; prompt investigation of newly discovered, potentially destructive plant diseases; research on diseases of new crops being grown to meet war-time shortages of oil, fibers, drugs, spices, etc.; more general study of soybean diseases; and work on effective home-made dusting, spraying and treating equipment where commercial equipment is unavailable.

The Society was urged to setup as a war measure a plan for cooperative preparation of annual summaries of progress in various fields of plant-disease research with the help of the subject-matter subcommittees under the Society's War Emergency Committee.

The subsequent general discussion brought out the fact that the Society's national and regional war emergency organization was well adapted for prompt exchange of research information. The necessity for adequate, coordinated plant-disease survey work was repeatedly emphasized. Helpfulness of specialists in performing identification for colleagues was commended. The *Plant Disease Reporter* was declared useful in facilitating such collaboration and for prompt dissemination of important new findings. Voluntary cooperation for adequate attack on many plant-disease problems was stressed. The tremendous national importance of plant-disease eradication and control programs was said to demand their maintenance at highest efficiency during the emergency.

Shortage of plant pathologists was declared to call for courage to discontinue work on diseases of plants not vital to the war effort to give more time for effective application of available research information. It was stated that growers of ornamentals would still demand assistance on their problems. Discontinuing fundamental studies, regardless of the plants involved, was held to be dangerous from the standpoint of the public interest.

It was generally agreed that basic research, designed to elucidate principles on which control measures must rest, is even more important in war-time than in peace-time, in order to avoid mistakes in the present and to lay a solid foundation for future procedures.

At a later session the need was discussed for research workers to assist in preparing an up-to-date, periodically revised manual of plant-disease control with recommendations for extension use throughout the country. Local pilot tests were held essential for successful local application of research findings and, also, for local education through their demonstration value. In a debate as to whether research, or extension workers should conduct such tests, it was claimed that in general the research man should, if possible, direct such work, but that highly effective results usually arose from cooperation between experiment station and extension service workers, including county agents.

Theoretically county agents are supposed to do a great deal of extension work in Plant Pathology. It is perfectly clear, however, that they have so many different kinds of things to do that relatively few of them are competent to play the part expected of them in disseminating information regarding plant disease control.

It was pointed out that losses from preventable diseases are still appalling. Epidemics often rage unchecked because proper control measures either are not taken at all —because they are not adequate, or because information regarding control measures had not been disseminated widely enough and at the proper time because of lack of sufficient trained personnel. One of the first and most important duties of plant pathology is prompt dissemination of information regarding the best available control measures. This responsibility cannot be discharged properly under present conditions. A survey of the situation with respect to extension plant pathologists in the country indicates that very few states have an adequate extension service. Some have no extension plant pathologist at all, and some of the most important agricultural states have but one such specialist, when two or three are needed. In only very few states can the situation with respect to extension work be considered satisfactory. Pathologists themselves are trying to do what they can by assembling and exchanging information, but the situation cannot be alleviated properly until more men are made available for this very important phase of insuring the Nation's supplies of essential materials from economic plants.

It was emphasized repeatedly that plant pathologists are finding difficulty in getting necessary things done because of the difficulty in retaining research assistants assigned to important problems. It was generally recognized that much research necessarily must be done by younger men, who are relatively free from distractions due to administrative work, public services and teaching. The national committee was instructed to study the situation and find out whether the special abilities of men subject to draft could not be used on important pathological problems, either in the places where they are now working, or in the armed forces. It was emphasized also that the depletion of the ranks of scientifically trained men constitutes a serious menace not only to present essential services to agriculture, but that it also jeopardizes the future of the discontinuance of certain basic researches that are essential in furnishing a basis for intelligent action in plant-disease control measures. Prospects are that there may be some alleviation of this situation.

Reports on ''Equipment priorities'' and ''The activities of the fungicide committee,'' given at the Summer Meetings, are appended.

EQUIPMENT PRIORITIES

Throughout the current year all permitted production of spraying and dusting machinery, as well as all farm equipment, has been on the basis of a quota based on the 1940 sales of the same type machines.

The 1942 per cent quota of 1940 for the period November 1, 1941 to October 31, 1942, and effective December 31, 1941 was as follows: Power Sprayers (not incl. engine)—97; Traction Sprayers—85; Hand Sprayers (capacity less 6 gal.)—100; Sprayers (capacity 6 gal. or more)—98; Spray Pumps, power—96; Power Dusters—103; Traction Dusters —94; Hand Dusters—100; Attachments and Parts (including repairs on sprayers and dusters)—140.

March 30, 1942, the above per cent quotas were revised in the case of Traction Dusting Machines and Hand Dusters to 103 for Traction Dusters and 110 for Hand Dusters.

Priorities made it possible to secure materials for production of the above quotas only. These priorities expire June 30.

Beginning July 1, 1942 production for next season goes on a quarterly basis and each manufacturer must submit to WPB a report which includes all the materials desired for production of machines during the quarter ending September 30. How much of this will be granted is, of course, not known.

Prior to November 1 the Department of Agriculture will advise WPB what crop production they desire for 1943. On the basis of this, WPB will then decide on and authorize a per cent quota of production of sprayers and dusters for 1943 use. There is no present knowledge on what this will be.

It is probable that in September a small per cent advance production of sprayers and dusters will be permitted. Whatever the total production authorized for 1943, a good production of repair parts probably will be permitted.

Canadian manufacturers of farm equipment have already been notified that their 1943 production will be on a basis sufficient to permit the manufacture of 25% of complete machines made by them in 1940, and 150% of repairs made during that year.

REPORT OF THE FUNGICIDE SUBCOMMITTEE

The committee was instructed (1) to procure information on supply and consumption of fungicides; (2) to seek priorities, if necessary; (3) to organize nation-wide research on substitutes and dosage.

At present the War Production Board lists copper and copper scrap at the top of the list of critically short materials. They write that estimated consumption exceeds supply by half. On the list also is formaldehyde, diphenyl amine, chlorinated hydrocarbons, chlorine and phenols. Clearly, these chemicals cannot be freely used in substitutes. The War Production Board lists borax, carbon tetrachloride, casein, mercury and zinc in the group of less critically essential materials.

All fungicidal materials have been given a blanket A10 priority. Some are assigned a higher rating. Mercury is limited to 80 per cent of 1941 and prohibited for turf fungicides and wood preservation. Despite its short position, copper will be available in nearly normal quantities for 1942.

Nineteen collaborators over the country have agreed to do research on dosage and substitutes.

Most pathologists, especially those who are familiar with manufacturing problems, agree that the ''best substitute for copper is copper.'' Therefore, the most productive research is to look into methods for ''stretching supplies.''

Some of the methods of stretching supplies to be investigated are: dilution, efficiency of application, particle size, and synergism with other compounds. Some of these factors have been studied in the past in relation to control. They should now be evaluated in terms of dose required for an equal level of control. Interactions of copper and sulphur have been studied. It was reported at the meeting that sulphur and cuprous oxide act synergistically not additively, so that the copper can be extended several fold.

Substitutes have been actively under study. On April 10 the committee mailed a list of 23 materials known to have fungicidal value. Since then a supplementary list of 265 organic compounds was investigated at New Haven under committee auspices. A tentative mimeographed report on these was distributed at Toledo. A more complete report will be given at the Winter Meetings at New York.

THE GERMINATION OF FUNGOUS SPORES IN RELATION TO CONTROLLED HUMIDITY

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Water, either liquid or vapor, has long been recognized as essential to the germination of spores of most pathogenic fungi. Furthermore, high atmospheric humidities are known to favor the initiation of infection of plants by fungous pathogens and the subsequent development of epiphytotics of plant diseases. However, fully convincing experimental data on the relation of high relative humidities to the germination of spores of most phytopathogenic fungi have been lacking, because of the difficulties of controlling the experimental conditions with such accuracy as to preclude the possible intervention of minute amounts of liquid water. The investigation reported herein was undertaken (1) to develop an accurate method for spore germination experiments at high relative humidities, and (2) to determine the relation of relative humidity to the germination of spores of certain phytopathogenic fungi.

LITERATURE REVIEW

Before humidity relations of fungi could be studied, suitable methods for controlling relative humidities had to be devised. In 1895 Lesage (18) made use of the vapor-pressure values of different concentrations of NaCl in one of the earliest attempts to determine the effect of relative humidity upon the germination of spores of *Penicillium glaucum*. The method for studying the humidity relations of fungi in culture by the use of aqueous solutions of H_2SO_4 was first described by Stevens (27) and further elaborated by Wilson (33). A laboratory method for maintaining constant humidity by means of saturated solutions of various chemicals was given by Spencer (26). Sweetman (29), in investigating the use of saturated solutions for the control of relative humidity in small closed containers, found that humidities obtained with certain saturated solutions did not change within 6 months. Critical discussions of the use of saturated salt solutions and of aqueous solutions of KOH, $CaCl_2 \cdot 6H_2O$, and H_2SO_4 to control the relative humidity have been presented by Hopp (16), Zwölfer (35), and others. Shaw (22) calculated from osmotic pressure values the relative humidities that solutions of sucrose of various weight-normal concentrations would provide at 25° C. Vernon and Whitby (32) pointed out that air, bubbled through a bottle full of water, is not necessarily saturated and that the method of controlling the humidity by mixing desiccated air with such presumably saturated air is faulty. One of the disadvantages of using H_2SO_4 is that SO_2 (35) or SO_3 (30) may be

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given off, especially if any organic material or particles drop into the solution.

Many observations and experiments on the relation of relative humidity to the germination of fungous spores have been made, chiefly, in conjunction with other lines of work, such as infection of plants, decay or deterioration of food products, and mildewing of textiles and book bindings. The data on the relation of relative humidity to spore germination in reports of such investigations cannot, in general, be considered highly accurate, because the conditions of the tests were not closely controlled, or, in many cases, they were not even stated. Such results may have been satisfactory for the purposes sought by the various investigators, but they can be considered merely indicative of the exact relation of humidity to the germination of fungous spores. In order to evaluate such data it is highly desirable to know the maximum fluctuation in the "constant" temperature at which tests are made.

Contact with water has been reported necessary to germination of all types of spores of many rust fungi; some experimental tests, however, have indicated that germination can occur in atmospheres just below saturation. Blackman (2) reported that teliospores of *Puccinia graminis, Phragmidium rubi,* and *Uromyces fabae* in a moist atmosphere germinated and formed normal sporidia, whereas, in hanging drops of water, spores produced long germ tubes that formed a few abnormal sporidia if the tubes came in contact with the air. Hirt (15) observed germination of teliospores of *Cronartium ribicola* on Ribes leaves and the formation and discharge of sporidia at relative humidities from 96 to 100 per cent. Sporidia of *Cronartium ribicola,* incubated on strips of cellophane for 48 hours at relative humidities ranging from 94 to 100 per cent, germinated abundantly at a humidity of 100 per cent, commonly at 99 per cent, and rarely at 97 per cent. Doran (8) stated that the aeciospores of *Gymnosporangium clavipes* germinated well on a dry slide in moist air. Smith (24) reported germination of urediospores of *Puccinia sorghi* on a dry substratum at relative humidities from 97.5 to 100 per cent. Stock (28) found that urediospores of *Puccinia triticina, P. dispersa, P. coronifera,* and *P. graminis* on dry slides failed to germinate at relative humidities below 99 per cent. Hemmi and Abe (14) stated that the average germination of urediospores of *Puccinia glumarum, P. triticina,* and *P. lolli* on dry slides at relative humidities of 100, 99, and 95 per cent, respectively, ranged between 12 and 40, 1.5 and 16, 0 and 0.5 per cent.

That conidia of some powdery mildew fungi would germinate on glass slides or leaves in moist air was mentioned as early as 1884 (25). Corner (7) mentioned that conidia of *Erysiphe graminis, Podosphaera leucotricha, Sphaerotheca pannosa, E. cichoracearum,* and *Oidium euonymi-japonici,* when immersed in water, produced a short germ tube or, commonly, none at all, whereas on dry glass in a saturated atmosphere they germinated readily. Berwith (1) concluded that a high atmospheric humidity was essential for the germination of the conidia of the apple powdery mildew fungus. Dundas (9) found that spores of *Erysiphe polygoni* from bean would germinate on dry slides at ordinary room humidity, as well as on water. Yarwood (34) reported that at favorable temperatures conidia of *Erysiphe polygoni* from red clover or bean germinated at relative humidities of 100 to approximately 0 per cent. Hashioka (13) reported that conidia of *Sphaerotheca fuliginea* germinated only in the saturated condition and were unable to germinate in relative humidities below 99 per cent. Longrée (19) made a careful and thorough study of the effect of temperature and relative humidity on the germination of conidia of *Sphaerotheca pannosa* var. *rosae*. In germination tests with conidia dusted on dry glass slides she experienced great difficulties in obtaining relative humidities close to 100 per cent without getting condensation. She accomplished it by means of a special humidity-control chamber in which temperature, and relative humidity, could be strictly controlled. The germination was excellent at a relative humidity of about 97 to about 99 per cent, somewhat lower at 99.8 to 100 per cent, very low at 95 per cent or below, and zero below 75 per cent. Germination of conidia dusted on leaves of rose shoots kept in a controlled environment was reduced as the atmospheric humidity of the environment was decreased; but, in general, spores on the surfaces of leaves germinated in an apparently very dry atmosphere. Brodie and Neufeld (3) reported germination of conidia of *Erysiphe polygoni* from *Delphinium* and from *Polygonum aviculare* and of *Erysiphe graminis* from *Poa pratensis* through a range of relative humidity from approximately zero to 100 per cent.

Guba (12) found that at least a relative humidity of 95 per cent was required for germination of the conidia of *Cladosporium fulvum* on dry glass.

Chowdhury (4, 5) attempted to determine the relation of relative humidity to the germination of spores of certain Indian fungi. Spores were placed on dry cellophane squares, previously soaked in 1 per cent glucose solution. The squares were then suspended over NaCl or H_2SO_4 solutions in stoppered bottles. His results indicated that the minimum relative humidity at which germination occurred was 90 per cent with spores of *Acrothecium penniseti*, *Alternaria brassicae*, and *Cladosporium herbarum;* 91 per cent with spores of *Helminthosporium frumentacei;* 93.9 per cent with spores of *Gloesporium tabernaemontanae*, and *Phyllosticta cajani;* and 95 per cent with spores of *Colletotrichum falcatum*, *C. lindemuthianum*, and *C. graminicolum*.

Tomkins (31), in tests at "known temperatures," observed spores of *Alternaria citri* germinating at relative humidities of 90.9 to 100 per cent. He concluded that the influence of the nature of the solid surfaces supporting the spores could be neglected, since the average lengths of germ tubes from spores on quartz, glass, Canada balsam, dammar, or shellac did not show any consistent differences.

Groom and Panisset (11) reported a minimum relative humidity of 81 per cent for germination of the spores of *Penicillium chrysogenum*.

Galloway (10) studied the moisture requirements of about 40 species of the more common mold fungi in reference to mildew in textiles. Spores were placed on dry squares of cellophane that had been soaked in dilute wort of 1 per cent maltose content. The squares were suspended over $CaCl_2$ solutions in stoppered bottles. On such nutritive substrata the lowest relative humidity at which germination occurred was between 75 and 90 per cent for different species of *Aspergillus*, 80 and 85 for species of *Penicillium*, 85 and 90 per cent for *Trichoderma*, 90 per cent for *Stemphylium*, *Cladosporium*, *Acrothecium*, or *Stachybotrys*, and 95 per cent for *Thielaviopsis*.

METHODS AND MATERIALS

Saturated aqueous salt or acid solutions, aqueous sucrose solutions, anhydrous $CaCl_2$, and redistilled water were used to control the relative humidity within sealed 1-quart glass fruit jars used as humidity chambers. Salts or oxalic acid of analytical reagent grade, C.P. analyzed sucrose, and redistilled water from a block-tin still were used in preparation of control solutions (Table 1). The chambers were thoroughly cleaned and rinsed with redistilled water. The rubber gaskets for the chambers were washed in CS_2 for 10 minutes and dried at least 24 hours before they were used to remove any sulphur present on their surface. The amount of solution, or water, used in each chamber was 250 cc. In the saturated solutions (26) an excess

TABLE 1.—*Composition and concentration of solutions used to provide the stated theoretical relative humidities within sealed glass chambers at 20° C.*

Material used	Concentration of solution	Theoretical relative humidity[a]
		Per cent
Redistilled water	....	100.0
Sucrose	0.10 molal	99.85
Sucrose	0.20 "	99.62
Sucrose	0.30 "	99.43
Sucrose	0.40 "	99.24
Sucrose	0.50 "	99.04
Sucrose	0.60 "	98.86
Sucrose	0.70 "	98.65
Sucrose	0.80 "	98.45
Sucrose	0.90 "	98.24
Sucrose	1.00 "	98.03
$H_2C_2O_4 \cdot 2H_2O$[b]	Saturated[c]	96.0
$Na_2HPO_4 \cdot 12H_2O$	"	95.0
$Na_2SO_4 \cdot 10H_2O$	"	93.0
K_2HPO_4	"	92.0
K_2CrO_4	"	88.0
$(NH_4)_2SO_4$	"	81.0
$Mg(C_2H_3O_2)_2 \cdot 4H_2O$	"	65.0
$Ca(NO_3)_2 \cdot 4H_2O$	"	55.0
$CaCl_2 \cdot 6H_2O$	"	32.0
$KC_2H_3O_2$	"	20.0
$CaCl_2$ (anhydrous)	....	0.0

[a] Calculations for sucrose solutions based on data from Morse, *et al.* (20) and data for saturated solutions taken from Spencer (26) except as indicated in [b].

[b] Based on Obermiller's (21) and Shaw's (22) measurements.

[c] An excess of solid phase was maintained in contact with the liquid phase of each saturated solution.

of the solid phase was always maintained. The theoretical relative humidities that various molal solutions of sucrose would provide in closed chambers were calculated by dividing the vapor pressures of the sucrose solutions by the vapor pressure of water at 20° C. The vapor pressures of the sucrose solutions were calculated from the osmotic pressure values (27).

After adding the required amount of sucrose and 230 cc. of redistilled water to each of the humidity chambers, they were sealed and steamed at 70° C. for 10 minutes to inactivate enzymes possibly present in the sucrose. After the chambers had cooled, 50 mg. of $K_2Cr_4O_7$, contained in 20 cc. of solution, were added to the sucrose solution in each chamber to prevent growth of organisms (6). Calculations showed that the $K_2Cr_4O_7$, assuming complete ionization, would theoretically lower the relative humidity within the chambers by 0.0003 per cent.

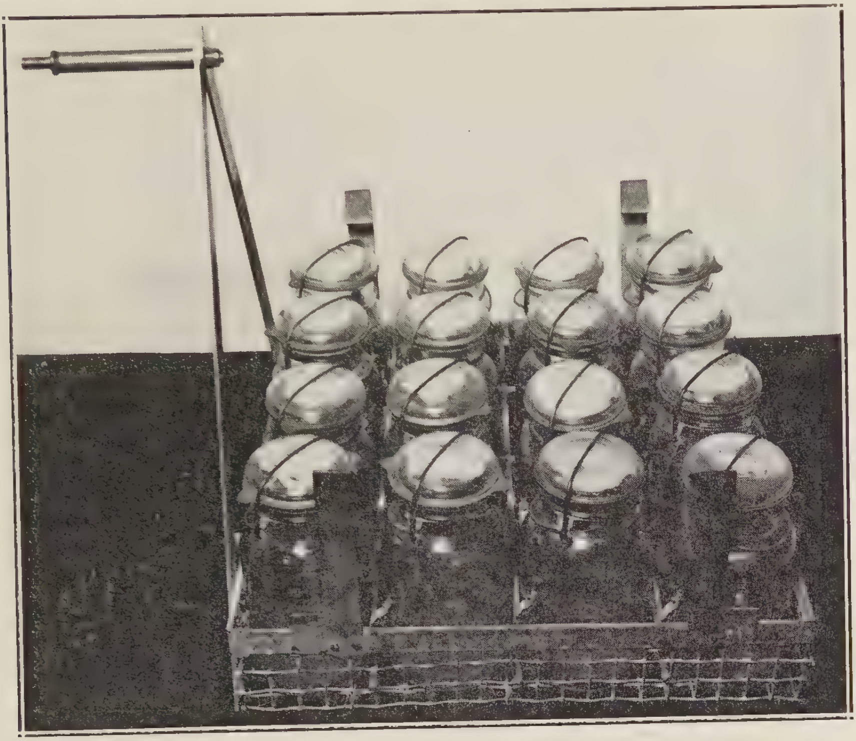

FIG. 1. Wire-mesh basket containing humidity chambers.

All experiments were made for 24, 48, or 72 hours in a water bath whose temperature was thermostatically controlled at 20° ± 0.02° C. During each series the temperature of the water bath, as indicated on a Beckmann thermometer that could be read to 0.01° C., was recorded at frequent intervals. The temperature of the water bath also was continuously recorded by means of a soil thermograph sensitive to changes of 0.2° C. or more. The room in which the experimental apparatus was assembled was kept at a temperature of 18°–20° C. and a relative humidity between 30 and 50 per cent.

To minimize stratification of the atmosphere or the solutions within the chambers the apparatus shown in figure 1 was used. The wire-mesh basket held 16 humidity chambers submerged in the water bath. The basket, on a brass rod support at its middle, was rocked to an angle of 17 degrees at a rate of 6 times a minute by means of a mechanical rocker arm connected to a gear reducer. An electric motor continuously operated the gear reducer and the water-bath agitator. The gentle rocking of the chambers caused no splashing of the solution within them.

Each series was maintained in the dark, exclusive of the short periods of diffuse light from the heating unit and the periods of several hours a day when a 150-watt electric bulb was used in the room in order that the necessary observations and experimental work could be performed. The exposure of the spores to rather diffuse light during these periods is believed to have been an insignificant factor affecting germination.

Experiments were made with conidia of *Sclerotinia fructicola* (Wint.) Rehm; ascospores and condia of *Venturia inaequalis* (Cke.) Wint.; urediospores of *Puccinia coronata* (Pers.) Cda., *P. graminis tritici* Eriks. and Henn., and *P. graminis avenae* Eriks. and Henn.; chlamydospores of *Ustilago hordei* (Pers.) K. and S. and *U. nuda* (Jens.) K. and S.; conidia of *Erysiphe polygoni* DC. from red clover (*Trifolium pratense* L.), cabbage (*Brassica oleracea* L.), and evening primrose (*Oenothera biennis* L.); and conidia of *E. graminis hordei* Marchal from barley (*Hordeum vulgare* L.) Precautions were taken to use as uniform spore material as was feasible.

Spores on or in drops of redistilled water on several clean cover glasses on glass slides in Petri-dish moist chambers at room temperature served as controls in each series.

Spores were dusted, brushed, or discharged naturally over the glass, paraffin, quartz, or leaf surfaces that served as spore-carriers (Fig. 2). Each carrier was then attached with paraffin to one end of a small glass rod. The other end of the rod was heated and imbedded in paraffin inside one of 4 Van Tieghem rings that had been sealed with paraffin to the inner part of a glass top of a humidity chamber. As many as 8 spore carriers could be attached to a single top within 2 minutes. This top was then substituted for one on a chamber in equilibrium with the water bath. The entire operation of removing a chamber, drying, making the substitution, and returning the chamber could be completed within 30 seconds.

Different solid substances were used as spore-carriers to determine (1) whether the tendency for condensation of water vapor on them varies to a degree sufficient to cause differences in germination at high relative humidities, or (2) whether differential effects might be exerted by minute amounts of soluble materials emanating from the spore-carriers. Non-nutritive spore-carriers were used, because the presence of extraneous materials on their surface probably would affect the relative humidity surrounding the spores. The glass surfaces were those of dry cover glasses that had been thoroughly cleaned in hot acid cleaning solution, rinsed several times in distilled water, placed over night in redistilled water, and dried with clean cheesecloth. The paraffin surfaces were those of cover glasses, thinly coated with imported white filtered paraffin with a high melting point, 68°–72° C. Prior to its use, the paraffin was kept at 85° C. for two weeks in order to rid it of any possible volatile materials. Clean pieces of fused or ground quartz were used in a limited number of series of experiments. The ventral leaf surfaces used in one series were portions of a large uninjured mature leaf from a potted Fameuse apple tree growing in the greenhouse. Disks 2.5 cm. in diameter were cut from the leaf with a cork borer. A disk was placed on each of several cover glasses with the dorsal surface adjacent to the glass. Paraffin was used to seal the margin of the disk to the surface of a cover glass. After 24 hours in the chambers the disks were cleared in a solution of glacial acetic acid-ethyl alcohol. Care was taken to avoid dislodging any of the spores during the clearing; they, however, adhered tenaciously and were not easily dislodged.

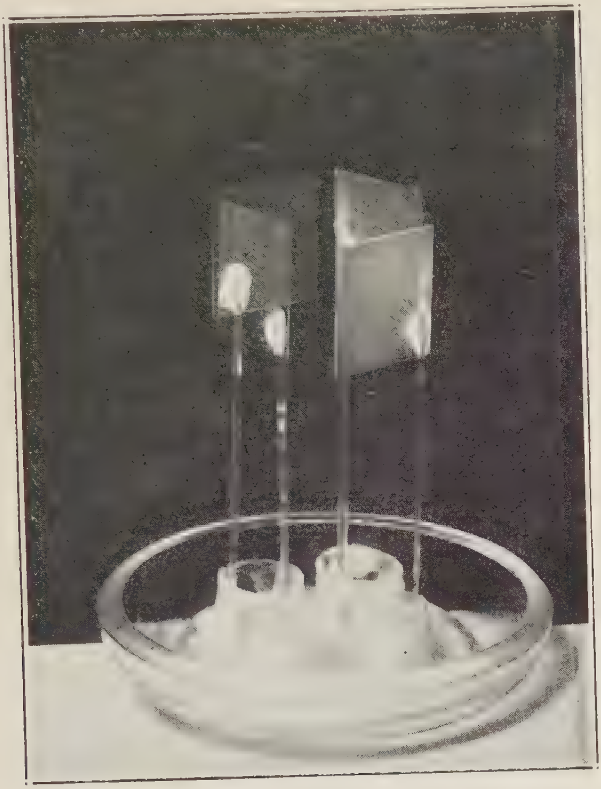

FIG. 2. Spore carriers attached by means of glass rods to the top of a humidity chamber.

To facilitate taking counts and to kill the spores at the end of each test, the spores usually were stained with aqueous crystal violet solution or with cotton blue in lactophenol. The spore carriers were mounted on slides, using a rosin-lanolin mixture (23) for sealing the margins. Spores carefully mounted in such manner remained in satisfactory condition for many months.

Experimental data were expressed in percentage germination and in length of germ tube. Counts of the number of germinated and nongerminated spores in 3 to 10, usually 5, random samples of 100 spores each were made on every spore-carrier. Replicate spore carriers of each type of surface and replicate chambers at each relative humidity were usually used in 2 to 8 series with spores of each fungus. Therefore, the total number of spores of a fungus that was counted at each relative humidity on the glass, paraffin, quartz, or leaf surface ranged between 300 and 50,000. Measurements of the length of germ tubes from 25 to 150 germinated spores taken at random were made at the various relative humidities in several series with most fungi used.

EXPERIMENTAL RESULTS

Conidia of Sclerotinia fructicola

Colonies of a monoconidial isolate of *Sclerotinia fructicola* were grown on a malt-agar medium in Petri dishes at 20° C. Tufts of conidia under 4 days of age were touched lightly with a camel-hair brush that had the hairs clipped short and of the same length. Extreme care was taken to avoid touching the surface of the agar medium with the brush. The spores adhering to the brush were dusted or brushed lightly over glass, paraffin, or quartz spore carriers used in tests at high relative humidities for periods as long as 72 hours.

The mean germination of conidia in redistilled water after 4 hours was 12 per cent and after 48 hours 87 per cent, the means in the different series ranging between 65 and 97 per cent. The mean length of germ tubes from conidia in water after 3, 4, 6, 7½, 11, 16, and 22 hours, respectively, was 25, 40, 72, 97, 148, 305, and 474 μ.

Single conidia on dry glass or paraffin did not germinate at relative humidities of 100 per cent or below in 2 experimental series for 24 hours, 3 for 48 hours, and 1 for 72 hours, respectively. Likewise, single condia on quartz at relative humidities of 100 per cent or below for 48 hours failed to germinate. In a few rare instances, where a little agar medium had been carried by the brush to the surfaces of the spore carriers, a few conidia germinated at high relative humidities.

Ascospores of Venturia inaequalis

Ascospores of *Venturia inaequalis* were discharged naturally from mature perithecia in apple leaves that had been soaked in water for a few minutes and placed on wet filter paper in glass dishes inverted over dry cover glasses. Precautions were taken against wetting of spores and cover glasses.

The mean germination of the ascospores on dry glass was high at a relative humidity of 100 per cent, low at 99 per cent, and zero at 98.7 per cent (Fig. 3, A). The mean percentage germination at a relative humidity of 100 per cent was slightly lower than that of controls in redistilled water in which the germination after 3, 4, 7, 16, 24, and 48 hours, respectively, was 36, 66, 73, 97, 96, and 97 per cent. The mean germination at a relative humidity of 100 per cent after 24 and 48 hours, respectively, was 85 ± 4 and 94 ± 2 per cent. At relative humidities of 99.85, 99.6, and 99.4 per cent the percentages of germination were progressively lower than at 100 per cent. The mean germination at a relative humidity of 99.6 per cent after 24 hours in 5 series was 28.2 ± 5.4 per cent. At a relative humidity of 99 per cent the mean germination in the various series ranged between 0 and 23 per cent, whereas no germination occurred at relative humidities of 98.7 per cent or below.

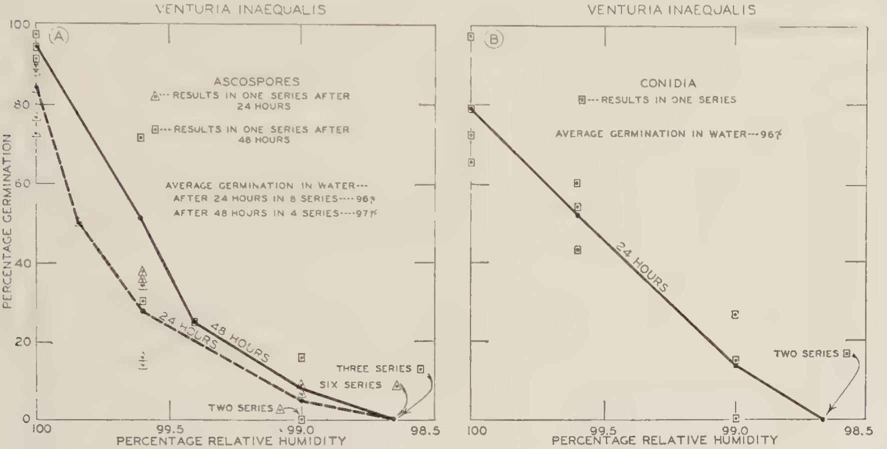

FIG. 3. Relation of relative humidity to germination of spores of *Venturia inaequalis* on glass at 20° ± 0.02° C. A. Ascospores. B. Conidia.

The mean length of 50 to 150 germ tubes from spores in water and at various relative humidities for 24 hours and 48 hours, respectively, are presented in figure 4. The relation of time to cumulative increase in length of germ tubes from spores in water also is shown. The mean length of the germ tubes was reduced with decreasing relative humidities. The mean length of the germ tubes at a relative humidity of 99.6 per cent was significantly less than at 100 per cent.

Comparative tests were made with ascospores of *Venturia inaequalis* on the surface of cover glasses, paraffin-coated cover glasses, and the ventral surface of a mature apple leaf. The experiments were made in an attempt to determine whether the chemical or physical properties of the spore-carriers might alter the relation of relative humidity to the germination of the spores. The number of spores counted on each surface at each relative humidity ranged between 300 and 1,700.

Results from the comparative use of different surfaces as spore-carriers

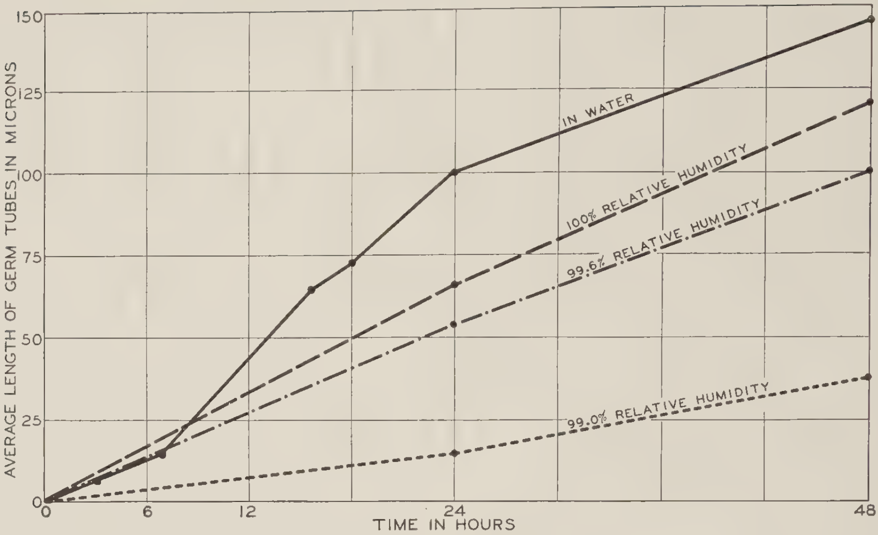

FIG. 4. Relation of water and relative humidity to length of germ tubes of ascospores of *Venturia inaequalis* on glass at 20° ± 0.02° C.

are shown in figure 5. The variations in the percentage germination on the three types of surfaces were relatively small. Germination did not occur at a relative humidity of 98.7 per cent on any of the surfaces. While these data are too limited to justify conclusions, they suggest that the different surfaces used did not exert any important influence on germination.

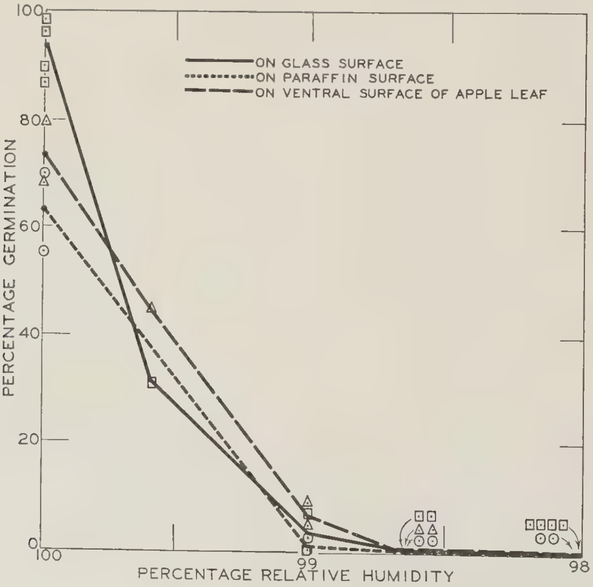

FIG. 5. Effect of various surfaces on the relation of relative humidity to germination of ascospores of *Venturia inequalis* at 20° ± 0.02° C.

Conidia of Venturia inaequalis

Conidia of *Venturia inaequalis* from scab lesions that had been macroscopically visible for 3 or 4 days were brushed lightly over dry cover glasses. The lesions had been produced on vigorous leaves of a potted Fameuse apple tree in the greenhouse. A monoascosporic culture was applied to the tree as inoculum. Two to 4 series were made at relative humidities from 98 to 100 per cent. The total number of spores counted at each relative humidity ranged between 1300 and 2700.

The results, graphically presented in figure 3, B, indicate that the conidia and ascospores of *Venturia inaequalis* have similar relative humidity requirements for germination. The mean germination of conidia in water and at relative humidities of 100, 99.6, 99.0, 98.7, and 98.0 per cent, respectively, was 96 ± 2, 79 ± 7, 52 ± 4, 14 ± 8, 0, and 0 per cent. The germination of the conidia was not quite so uniform as that of the ascospores. However, it is noteworthy that neither conidia nor ascospores on dry glass germinated at relative humidities of 98.7 per cent and below.

Conidia in redistilled water for 4 hours had germ tubes 10 to 15 μ in length. The germination of conidia after 5½ hours in water was 85 per cent, the average length of germ tubes being 20 μ. The mean length of 25 germ tubes from spores in water for 24 hours was 146 μ.

Two comparative series of experiments for 24 hours were made with *dry* conidia brushed from leaf lesions over cover glasses and with *wet* conidia obtained in suspension from leaf lesions and atomized over cover glasses. The water on the cover glasses with the *wet* conidia was dried in an air stream. The *wet* condia were in water for a total period of 30 to 40 minutes before they were dried. At relative humidities of 100, 99.6, 99.0, 98.0, and 92.0 per cent, respectively, the mean germination of the *wet* conidia was 74, 67, 9, 0, and 0 per cent, whereas that of the *dry* conidia, at the same relative humidities, was 79, 52, 14, 0, and 0 per cent. Evidently, the wetting of the conidia for 30 or 40 minutes did not appreciably affect either the minimum relative humidity at which germination would occur or the mean percentage germination at relative humidities from 99 to 100 per cent.

Urediospores of Puccinia coronata, P. graminis tritici, and P. graminis avenae

Spore material of *Puccinia coronata* collected in Wisconsin and of *P. graminis tritici* race 56 and *P. graminis avenae* race 2 received from E. C. Stakman served to establish these fungi on susceptible plants on which inoculum could be maintained. Subsequent inoculations were made on State's Pride (Wis. Ped. No. 7–7) oat seedlings or on Marquis spring-wheat seedlings growing in the greenhouse. Urediospores were shaken or brushed lightly over a watch glass from uredia that had been macroscopically visible for 3 to 7 days on vigorous leaves. The spores were mixed lightly with the camel-hair brush with which they were dusted over the glass or paraffin spore carriers. One to 4 series for 24 hours and 1 to 2 for 48 were made with spores of each fungus at humidities ranging from 92 to 100 per cent. A total of 500 to 5,000 spores was counted for each relative humidity on each type of spore carrier.

The mean germination of spores of *Puccinia coronata* on or in thin drops of water on cover glasses was 83 per cent after 24 hours and 89 per cent after 48 hours. In one experiment the mean germination in water after 4½ hours was 95 per cent. The germination on glass at a relative humidity of 100 per cent was considerably lower than in water. At a relative humidity of 99 per cent the average germination on glass was 15 per cent after 24 hours and 32 per cent after 48 hours. None of the spores on paraffin for 48 hours and only 0.2 per cent of those on glass germinated at a relative humidity of 98 per cent. Spores on either glass or paraffin exposed to relative humidities of 95 or 92 per cent for 48 hours failed to germinate. The percentages of germination of spores on paraffin at relative humidities of 99 or 100 per cent were somewhat lower than those on glass.

The mean length of the germ tubes from spores of *Puccinia coronata* on or in a thin drop of redistilled water was 192 μ after 4½ hours, 273 μ after 5½ hours, and 468 μ after 9 hours. The average lengths of the germ tubes after 24 hours on glass at relative humidities of 100, 99, 98.7, and 98 per cent, respectively, were approximately 555, 289, 172, and 76 μ. The average length of the germ tubes after 48 hours was not appreciably greater than after 24 hours. The germ tubes at relative humidities of 100 per cent or below did not show nearly so much of the knotty or branching tendency as did the germ tubes of *P. graminis* at the same relative humidities.

Spores of *P. graminis tritici* placed on or in redistilled water for 6 hours exhibited from 95 to 100 per cent germination. The mean germination on or in water on glass after 24 and 48 hours, respectively, was 98 and 97 per cent and on paraffin after 24 hours was 99 per cent. Germination of spores in water started before 2 hours had elapsed. The mean germination on glass at a relative humidity of 100 per cent was 81 per cent after 24 hours and 86 per cent after 48. The percentage germination at a relative humidity of 99 per cent was much less than that at 100, whereas, at 98 per cent, the percentage germination was very small, being zero in some series. At a relative humidity of 95 per cent a few spores had very short germ tubes in one test on glass for 24 hours and in a test on paraffin for 48 hours. The percentage germination at a relative humidity of 100 per cent was slightly higher on paraffin than on glass, but the variations in percentages of germination between the two surfaces in different series were not always consistent. Therefore, it is believed that the differences in the percentages of germination between glass and paraffin at 100 per cent relative humidity were not significant. At relative humidities of 99 and 98 per cent in tests for 24 and 48 hours, the percentage germination on glass was slightly higher than on paraffin.

The mean length of germ tubes from spores in water after 2, 5, 6, 12, and 48 hours, respectively, was 80, 200, 247, 654, and 590 μ. The germ tubes of spores on dry glass at relative humidities of 100 per cent or less were very knotty or branched, whereas, in water, the germ tubes were only slightly knotted. Length of germ tubes decreased in direct relation to decrease in relative humidity.

The results with spores of *Puccinia graminis avenae* were similar to those with *P. graminis tritici*. Spores on or in redistilled water for 9 hours produced germ tubes averaging 390 μ in length. Those in water on glass for 24 or 48 hours, respectively, showed 87 and 99 per cent germination. The percentage germination of spores on glass at a relative humidity of 100 per cent was somewhat lower than in redistilled water, and at 98 per cent was very low. Germination on paraffin was, at most relative humidities, somewhat lower than on glass. The mean lengths of the germ tubes from spores of *P. graminis avenae* after 24 hours at relative humidities of 99 and 98 per cent, respectively, were 150 and 58 μ.

A comparative series of tests with urediospores of *Puccinia coronata*, *P. graminis tritici*, and *P. graminis avenae* was made. A glass and a paraffin spore carrier for each of the 3 fungi were placed in each of 4 humidity chambers at each of 4 relative humidities; namely, 100, 99, 98, and 95 per cent. Two of the chambers at each relative humidity were used in a 24-hour series and the other two were allowed to continue in a 48-hour series. The number of spores counted on most of the spore carriers was 500, ranging between 300 and 1000. The results (Fig. 6) indicate that the germination of the urediospores of *P. coronata* is slightly lower than that of the two stem rust fungi. The urediospores of *P. coronata* did not germinate at relative humidities of 98 per cent or below, whereas a low percentage of the urediospores of *P. graminis tritici* and *P. graminis avenae* did germinate at a relative humidity of 98 per cent.

Chlamydospores of Ustilago hordei and U. nuda

The experiments with *Ustilago hordei* were made with chlamydospores that were brushed from a few smutted heads of barley into a vial where they were kept until used. In preliminary tests germination of the 6- to 8-month-old spores on water ranged between 30 and 57 per cent. Several series for 48 hours were made on glass and paraffin at 8 relative humidities between 81 and 100 per cent. A total of 1000 to 9000 spores was counted at each relative humidity.

The results of two representative series are presented graphically in figure 7, A. In the controls spores on redistilled water started to germinate within 3 hours and germination after 48 hours on redistilled water on glass or paraffin was approximately 40 per cent. The mean germination on glass at a relative humidity of 100 per cent was less than half that on redistilled water. At a relative humidity of 99 per cent the mean germination was only slightly lower than at 100 per cent. The mean percentages of germination on glass at relative humidities of 95, 98, 99, and 100 per cent, respec-

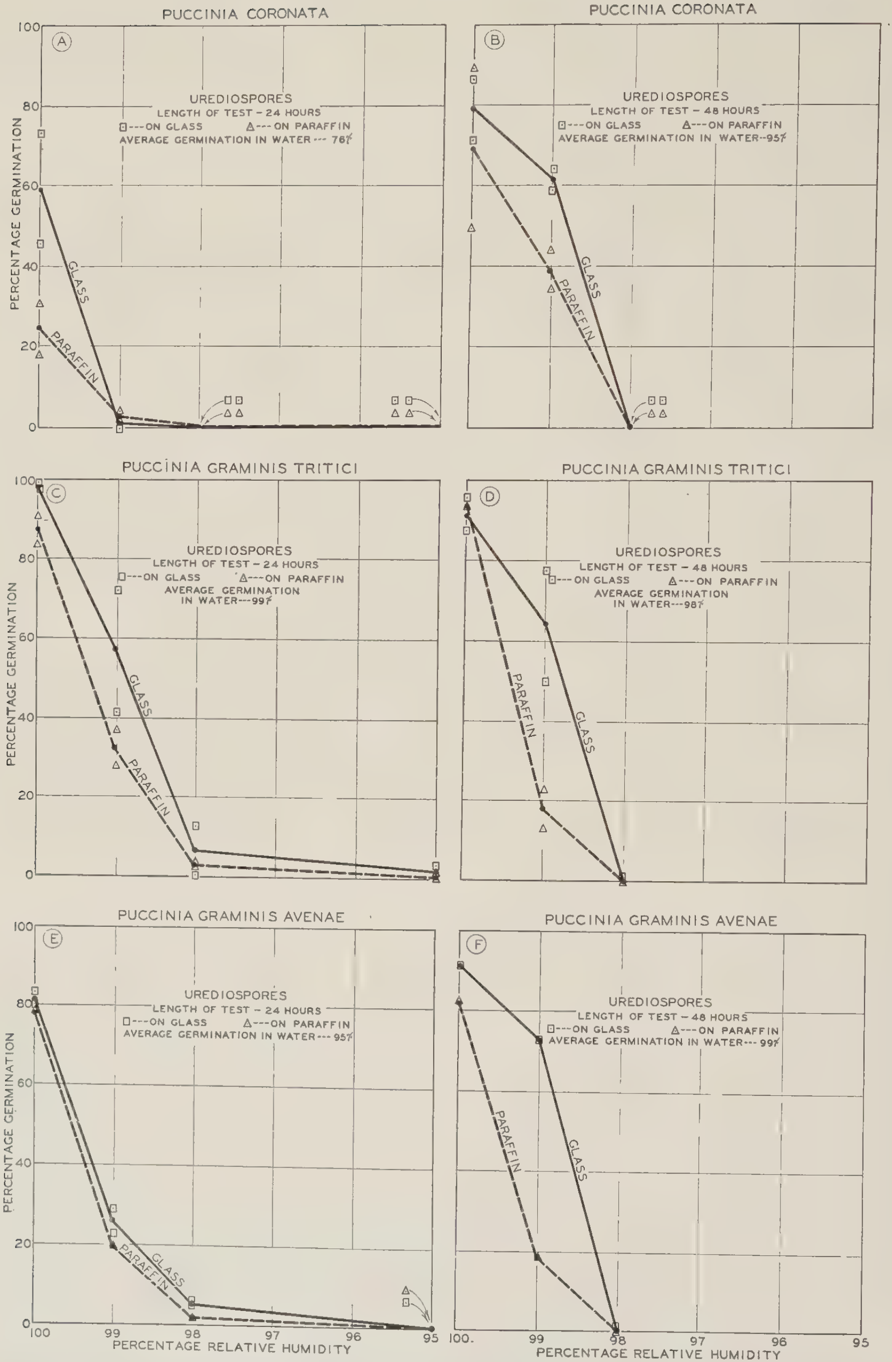

FIG. 6. Relation of relative humidity to germination of urediospores on glass and paraffin in a single series at 20° ± 0.02° C. A, B. *Puccinia coronata.* C, D. *P. graminis tritici* (race 56). E, F. *P. graminis avenae* (race 2).

tively, were almost always higher than on paraffin. Spores on glass or paraffin failed to germinate at a relative humidity of 93 per cent or below.

Production of sporidia was fairly low on redistilled water, very sparse at a relative humidity of 100 per cent, and absent at 99 per cent or below.

Long primary basidial hyphae grew from the basidia at relative humidities of 100, 99, 98, or 95 per cent. The average lengths of the basidia, together with the longest basidial hyphae at a relative humidity of 100 per cent after 24, 27, 35, and 48 hours, respectively, were 62, 81, 150 and 217 μ. The basidial hyphae at the lower relative humidities at which germination occurred were only slightly shorter than those at the higher relative humidities.

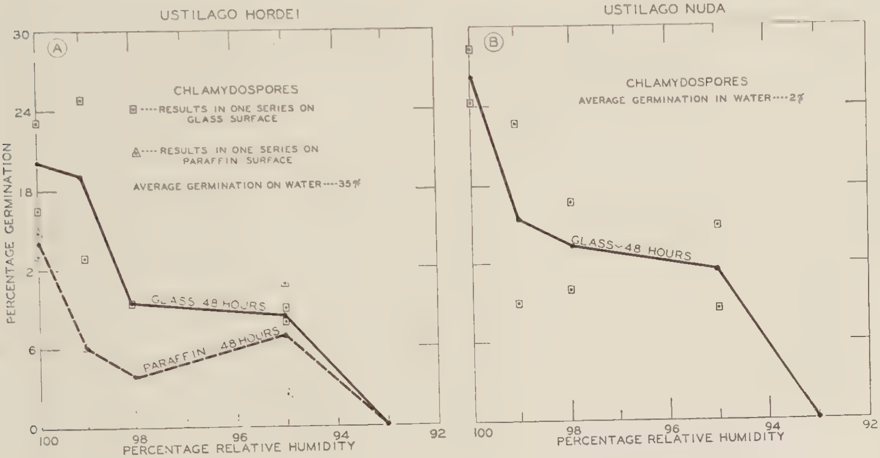

FIG. 7. Relation of relative humidity to germination of chlamydospores at 20° ± 0.02° C. A. *Ustilago hordei.* B. *U. nuda.*

The chlamydospores of *Ustilago nuda* were obtained from a few smutted heads of barley of Hybrid × 163. The germination of the chlamydospores in or on water in preliminary tests ranged between 1 and 13 per cent. Two series were made for 48 hours on glass at various relative humidities from 88 to 100 per cent. The total number of spores counted at each relative humidity ranged between 2,000 and 23,000.

The results of the experiments on spores of *Ustilago nuda* are given in figure 7, B. In the controls the average germination was 2 per cent in redistilled water and 7 per cent on the surface of it. On dry glass at relative humidities from 100 to 95 per cent the percentage germination was higher than on water. The percentage germination at a relative humidity of 100 per cent was significantly greater than at 98, whereas it was lower at 95 than at 99 per cent. At 93 per cent relative humidity or below no germination occurred in two series of experiments.

The germ tubes from spores in water were relatively short; whereas, at relative humidities of 100, 99, 98, or 95 per cent, they were very long. The average length of 25 germ tubes from spores in water and at a relative humidity of 95 per cent, respectively, for 48 hours was 33 and 222 μ.

Conidia of Erysiphe polygoni from Red Clover, Cabbage, Evening Primrose and E. graminis hordei

Conidia of *Erysiphe polygoni* from young sporulating lesions on young, fully expanded leaves of red clover growing in the greenhouse were shaken over a watch glass. The spores were stirred lightly with the camel-hair brush with which the conidia were later dusted over clean, dry cover glasses. These spore carriers were promptly placed at various relative humidities from 0 to 100 per cent. It should be noted that the conidia on mildew lesions from which spores were to be obtained were brushed off 24 hours prior to an experimental series. Germination at the start of the tests was almost invariably zero and was never more than 1 per cent.

Experiments were made with conidia collected in the early afternoon on bright sunny days. The results from a representative series are presented graphically in figure 8, A. The mean length of 75 germ tubes of conidia of *Erysiphe polygoni* at each of the various relative humidities is shown in figure 8, B.

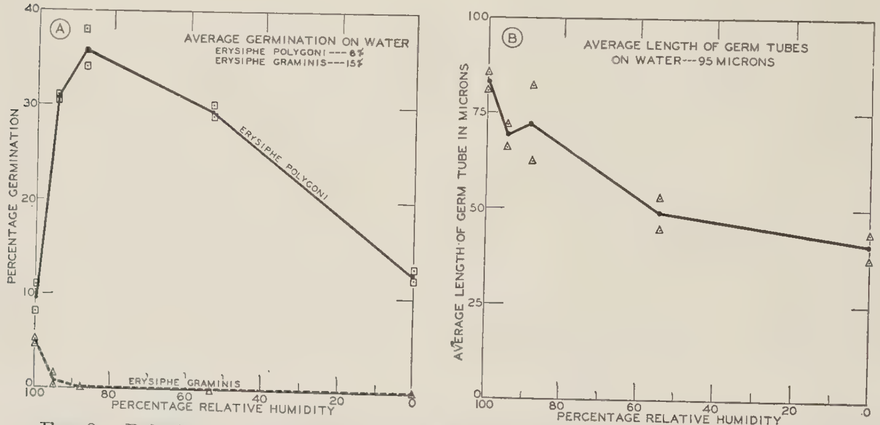

FIG. 8. Relation of relative humidity to germination of conidia of *Erysiphe polygoni* from red clover and of *E. graminis hordei* in a single series on glass at 20° ± 0.02° C. for 24 hours. A. Percentage germination. B. Average length of germ tubes of *E. polygoni.*

Germination of the spores on water was much lower than at high relative humidities. The percentage germination at relative humidities of 98, 95, or 88 per cent was usually higher than at 55 or 0 per cent.

The germ tubes from spores at most relative humidities were relatively short in comparison to the length of the conidia. The average length of 75 germ tubes from conidia after 24 hours at relative humidities of 0, 55, 88, 95, 96, and 100 per cent, and on water, respectively, was 37, 48, 75, 78, 81, 91, and 102 μ. It is evident, therefore, that germ-tube length was in direct relation to relative humidity.

The percentage of shriveled or dead spores at the beginning of the tests varied widely in different series. In one series, 21 per cent of the spores were shriveled at the start. At the end of the 24-hour series, the percentage of the spores shriveled at relative humidities of 0, 55, 88, 96, and 100 per cent, and on water, respectively, was 100, 82, 72, 63, 45, and 17. The vitality of many of the nonshriveled, apparently viable conidia was low, for they failed to germinate at the specific relative humidities.

Conidia submerged in water usually failed to germinate. The walls of many of them were ruptured, and the contents of most of the others had become granular or disorganized.

The spores, after dislodgment, are very short-lived under most conditions. The percentage germination in most series was less than 50 per cent at the most favorable relative humidity. In one series in which conidia were collected following 3 days of sunless cloudy weather, germination at all humidities from 0 to 100 was less than 10 per cent. The germinability of conidia in a sample seems influenced greatly by age of lesions and by temperature, moisture, and light conditions during the few days before sampling.

In two series of experiments with conidia of *Erysiphe polygoni* from cabbage, the relation of relative humidity to percentage germination proved similar to that of conidia of *E. polygoni* from clover. The average germination at relative humidities of 0, 20, 32, 55, 81, 95, and 100 ranged from 14 to 39 per cent. No consistent differences in the percentage germination were found at any of the relative humidities from 0 to 100 per cent, although there seemed to be a tendency for the percentage germination to be slightly higher at the higher relative humidities than over anhydrous $CaCl_2$. The germination in hanging drops of distilled water ranged from 1 to 6 per cent. Spores on dry glass slides exposed to the 35–50 per cent relative humidity in the laboratory for 22 hours showed 35 per cent germination.

In a 21-hour series the average germination of 500 to 1,000 conidia of *Erysiphe polygoni* from evening primrose at relative humidities of 100, 95, 88, 55, and 0 per cent, respectively, was 33, 24, 21, 5, and 9 per cent. At the start of the series, 51 per cent of the spores were shriveled and 1 per cent had germinated. Spores on dry glass slides, exposed to laboratory atmosphere for 21 hours, averaged 15 per cent germination.

Two series of experiments were made with conidia of *E. graminis hordei* from greenhouse-grown barley (C.I. 934) plants. The spores were shaken over a watch glass from leaves on which the mildew lesions had been macroscopically visible 3 days. The spores after being stirred on the watch glass were dusted over dry cover glasses. From 2,000 to 4,000 conidia were counted at each relative humidity.

The results from a representative series are shown in figure 8, A. The variation in results from the two series was relatively small. The average germination on water after 24 hours was 14.5 per cent, the germ tubes from some spores being 184 μ long. The mean length of 25 germ tubes from spores on water for 6, 25, and 54 hours, respectively, was 30, 139, and 130 μ.

The average germination at relative humidities of 100 and 95 per cent, respectively, was 5 and 0.8 per cent. The spores of this series showed almost no germination at relative humidities of 88 per cent or below. The length of the germ tubes at a relative humidity of 95 per cent was much less than at a relative humidity of 100 per cent.

DISCUSSION

The method applied in the spore-germination tests at high relative humidities, as herein recorded has the following advantages: (1) relative humidity and temperature are accurately controlled, (2) the variables to which spores are subjected are reduced to the relative humidity and the nature of the spore carriers, (3) a large number of tests can be made in a single series, and (4) the apparatus is relatively inexpensive.

Probably the most satisfactory and reliable method of controlling relative humidity for spore germination tests is obtained through aqueous solutions of pure nonvolatile chemicals inside a closed system maintained at an accurately controlled temperature. However, to prevent stratification of the solution or the atmosphere within the chamber or system and to increase the diffusion of water vapor throughout the chamber, it is desirable to use a small chamber and to agitate both solution and atmosphere by some such device as is described in the foregoing. The relative humidity in a closed system over a saturated salt solution or a chemical solution of a certain concentration at a stated temperature remains constant, provided the temperature does not vary and changes within the solution do not occur. However, with fluctuations in the temperature, equilibrium is disturbed and considerable changes in the relative humidity may occur before equilibrium is restored. Quick changes in temperature may produce abrupt changes in relative humidity, while the effect of a very small, gradual drift in temperature may be almost nullified by action of the control solution. The time necessary for the solution and atmosphere to reach equilibrium following changes in the temperature may be reduced by movement of the solution and atmosphere within the system. Fluctuations of temperature must be minimized if control of relative humidities close to 100 per cent is to be accurately maintained.

The slight temperature fluctuations of 0.02° C. within the water bath usually occurred gradually over a period of several hours. Therefore, the temperature changes at the surface of the spore carriers within the chamber must have been more gradual and of less magnitude than were the recorded temperature changes for the water bath. Since temperature changes within the chambers must have been extremely slight, the relative humidities must have been rigidly controlled.

Direct measurements of relative humidity were not made because of lack of a feasible method for making accurate determinations of high relative humidities within small closed chambers. However, spore germination served as a very delicate biological indicator of accuracy of control of the high relative humidities employed. Conidia of *Sclerotinia fructicola*, for example, germinated freely in water but did not germinate at a relative humidity of 100 per cent; and ascospores of *Venturia inaequalis* showed significantly different percentages of germination and lengths of germ tube at each of the following relative humidities: 100, 99.85, 99.6, 99.0, and 98.7 per cent.

It is recognized that in the tests in which the theoretical relative humidity is herein reported as 100 per cent, condensation was not always avoided. When it could be observed, it was only in a very thin film of precipitated moisture that was evanescent when the 100 per cent chamber was opened. However, germination of spores at this humidity differed consistently from that on or in drops of water. No visible condensation on the spore-carrying surfaces occurred at relative humidities of 99.85 per cent or below.

The question as to whether the nature of the spore carriers might affect the relation of relative humidity to germination was investigated by using glass, paraffin, and quartz in comparative tests with spores of several fungi. Since these substances have different physical and chemical properties, the tendency for condensation of water vapor on one might be greater than on another. The average percentage germination of spores of 3 species of cereal rust fungi, of *Ustilago hordei*, and of *Venturia inaequalis* was lower in most tests on paraffin than on glass. Yet, no great differences in results on these spore carriers were evident, for the germination of conidia of *Sclerotinia fructicola* did not occur on glass, paraffin, or quartz at relative humidities of 100 per cent or below. Since the percentages of germination on glass were usually slightly higher and more uniform than on paraffin and because glass was more convenient to use than paraffin or quartz, clean dry glass was employed in the greater portion of the germination studies.

Spores of various phytopathogenic fungi on the nonnutritive substrata used appeared to vary greatly in their ability to germinate at different relative humidities or in the absence of liquid water. The conidia of *Sclerotinia fructicola* on a nonnutritive surface apparently required contact with liquid water before they were able to germinate. On the other hand, the germination of ascospores and conidia of *Venturia inaequalis* was very high at a relative humidity of 100 per cent, medium at 99.6 per cent, low at 99.0 per cent, and zero at 98.7 per cent. Prior to the tests reported herein, it had not been demonstrated that the spores of the apple scab fungus could germinate at high relative humidities in the absence of liquid water. The minimum relative humidity for germination of urediospores of *Puccinia coronata* appeared to be at or slightly above 98.0 per cent, whereas, for urediospores of *P. graminis tritici* (race 56) and *P. graminis avenae* (race 2), it apparently was at or slightly below 98.0 per cent. The germination of some chlamydospores of *Ustilago hordei* and *U. nuda* occurred at a relative humidity of 95 per cent, but not at 93 per cent. Conidia of *Erysiphe polygoni* from red clover, cabbage, and evening primrose on glass germinated at all the relative humidities tested from 100 per cent to approximately 0 per cent. Conidia of *E. graminis hordei* appeared to be much more sensitive to moderate and low humidities than did the conidia of *E. polygoni*.

It is evident that spores of different fungi vary in their moisture requirements for germination. To explain these variations more knowledge concerning the fundamental mechanism, organization and nature of the membranes and protoplasts of a spore is needed. The membranes of different spores of the same species, as well as of different fungi, probably are extremely variable. The reserves in spores of different fungi vary greatly in composition and quantity and doubtless also, in their utilization of water. In rust spores, the reserves may be largely oils, whereas in conidia of the powdery-mildew fungi there may be stored materials of other types. Corner (7) suggested that the conidia of powdery-mildew fungi probably carry the water for germination within the large vacuoles in the spores. Brodie and Neufeld (3) stated that there is probably very little water present in the conidium of *Erysiphe polygoni*, the protoplast consisting of a gel-like material. Upon exposure of the papilla of the conidium to air, CO_2 would be released and respiration would begin. As a result, the viscid protoplast would be converted into materials more labile and voluminous, and the increase in volume necessary for the production of a germ tube might come from this source alone. The wettability of the spore wall and its permeability to water may be very important factors in determining the relation of relative humidity to germination.

Humidity is an important factor affecting incitation and development of plant diseases, especially those caused by pathogenic fungi. Epidemics of brown rot of fruits, caused by *Sclerotinia fructicola* and related species, follow periods of wet, rainy weather, since the spores require contact with water for germination. Apple scab (*Venturia inaequalis*) results from infections during or immediately following rain periods, free water, primarily rain, being essential to the discharge of ascospores and abscission and dissemination of conidia. However, at or near the optimum temperature, some of the spores can germinate at relative humidities of 99 per cent or above, and most of the spores germinate at a relative humidity of 100 per cent or in water. Many investigators have stated that water on cereal plants was required for their infection by urediospores of various species of *Puccinia*. Lauritzen (17) and others, however, have reported that the presence of water on the leaves was not necessary, because relative humidities approaching saturation were sufficient to permit infection. It was found in the present work that the fresh urediospores of *P. coronata*, *P. graminis tritici*, and *P. graminis avenae* on glass or paraffin germinated at relative humidities as low as 98 per cent in the absence of water, although contact with water appeared to be optimum for germination. Several workers have shown that heavier infection with some smut fungi occurs in medium dry than in wet soil. In the study reported here, it was demonstrated that chlamydospores of *Ustilago nuda* and *U. hordei* germinated at relative humidities of 95 to 100 per cent. Chlamydospores in water germinated poorly, whereas, on the surface of water, germination was fair. Thus, the heavier smut infection at medium to low soil moistures may be influenced by the ability of the spores to germinate at relative humidities below saturation. Infection with spores of many powdery mildews is readily accomplished on leaves of plants that do not become wet with water. That is possible because the conidia are able to germinate at relative humidities considerably below 100 per cent.

SUMMARY

A method for accurately controlling high relative humidities for spore germination tests was developed. Spores were discharged naturally or dusted with camel-hair brush over clean dry spore carriers. The carriers were then suspended in sealed glass chambers over sucrose solutions, saturated salt solutions, or redistilled water, the liquids used to secure the desired relative humidities, which were computed theoretically. The humidity chambers were placed in a wire container and completely submerged in a water bath the temperature of which was thermostatically maintained at 20.0° ± 0.02° C. Stratification of the atmospheres or the solutions within the chambers was minimized by mechanically rocking the container of the chambers. Visible condensation upon the spore-carriers was precluded except to a limited degree at a relative humidity of 100 per cent.

Conidia of *Sclerotinia fructicola* germinated well in redistilled water, but failed to germinate in tests for 72 hours on glass, paraffin, or quartz at relative humidities of 100 per cent or below.

It was demonstrated for the first time that ascospores and condia of *Venturia inaequalis* could germinate on dry glass at relative humidities of 99 to 100 per cent.

The mean percentage germination of urediospores of *Puccinia coronata*, of *P. graminis tritici* (race 56), and of *P. graminis avenae* (race 2) on glass was high in water, lower at a relative humidity of 100 per cent, considerably lower at 99.0, and practically zero at 98 per cent.

It was demonstrated for the first time that chlamydospores of *Ustilago hordei* and *U. nuda* on dry glass or paraffin could germinate at relative humidities of 95 to 100 per cent but not at 93 per cent or below. Long basidial hyphae of *U. hordei* were produced at the relative humidities of 100 to 95 per cent whereas sporidia were produced from basidia of spores germinating on water.

Conidia of *Erysiphe polygoni* from red clover, cabbage, and evening primrose on dry glass germinated at relative humidities from 0 to 100 per cent. However, the germ tubes were very short and survived for a very short period of time at low relative humidities.

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THE VALUE OF SPERGON AS A SEED PROTECTANT FOR CANNING PEAS[1]

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INTRODUCTION

The pea-canning industry in Minnesota has in the past 4 decades developed into a large and nationally important business, with a total production in 1941 of 27,470 tons. This investigation was undertaken to obtain information on the damage done to the Minnesota pea crop by root rotting fungi, and to determine the value of certain chemicals as seed protectants. Research initiated at the New York Agricultural Experiment Station (Geneva) indicated that certain synthetic organic compounds might have some value as seed protectants for canning crops.[2] From several synthetic compounds, a material was selected on the basis of laboratory and greenhouse assay as having fungicidal value, and was thought to show some promise as a seed protectant. This material, originally known as United States Rubber material #120, has recently been named "Spergon," and is a complex, synthetic organic chemical containing no copper, sulphur, mercury or zinc. It is a light greenish-yellow, free flowing powder, relatively insoluble in water, and contains a buffering agent to permit its use in alkaline soils.[3] In extensive laboratory and greenhouse experiments it has been found highly fungicidal,[4] and, in field experiments with 25 varieties of vegetables and ornamental plants, has caused no seed injury except on peppers, egg plants, and garden beets, even when applied in excess.

METHODS

In the spring of 1940, cooperative seed-treatment experiments were established with canneries in 7 localities in southern Minnesota. Samples of Spergon, sufficient to treat enough seed for planting 1 acre, were mailed to each cooperator, who then treated his seed with this material at the rate of 2 ounces to the bushel. The treated seeds were then planted in 1-acre plots in the same field and contiguous to non-treated or other comparable plots. Attempts were made to maintain a uniform seeding rate in each section of the experimental field, although different rates of seeding were used by the different cooperators. The rates of seeding at the different localities ranged from 4 to 4½ bushels of seed per acre. The investigation was continued in 1941, but at this time the experiments were confined to 1 cooperator, and plots of 10 acres for each treatment were established in a very large field of exceptionally uniform topography and soil type. Each plot was visited by one of the investigators at regular intervals, after planting, to obtain emergence data. Final stand counts, root-rot records, and yield data were obtained at harvest time.

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[1] Investigation made under the terms of a research fellowship sponsored by the United States Rubber Company, Passaic, New Jersey. Paper No. 1973, Scientific Journal Series of the Minnesota Agricultural Experiment Station.

[2] Cunningham, H. S., and E. G. Sharvelle. Organic seed protectants for lima beans. Phytopath. 30: 4. 1940.

[3] Spergon chemically is "Tetrachloro-para-benzoquinone."

[4] Felix, E. L. Tetrachloro para benzoquinone, an effective organic seed protectant. (Abstract) Phytopath. 32: 6. 1942.

Four criteria (stand of plants, disease rating, height of plants, and yields) were used at each location for comparing the Spergon-treated plots with the non-treated or other comparable plots.

a. *Stand of Plants* was determined by counting the number of plants within a measured area of 1 sq. yd., 10 such areas being selected at random throughout the plot. The average of these counts was used as the basis of comparison for the stand of plants in the different plots, the stand being expressed as the average number of plants per sq. yd.

b. *Disease Rating* was obtained by digging 50 plants at random from each plot. The entire sample was then examined immediately and the individual plants placed in 1 of 5 arbitrary classes, according to the severity of disease, as follows: 0, no infection; 1, slight discoloration of the stem; 2, discolored area diffused with slight necrosis; 3, marked discoloration, with well-defined necrosis; 4, stem or root completely girdled. The average of these 50 determinations was then used as the criterion for the severity of root rot in each plot.

c. *Height of Plants.* The 50 plants from each plot used in the previous determination of disease severity were measured, the length of roots and length of vine from crown to tip being recorded separately, the average of these determinations serving as the basis of comparison for the height of plants in each plot.

d. *Yield.* Each plot was accurately measured with a land wheel, the cutting of each plot was supervised by one of the writers and the yields were obtained directly from the viner in each location. Yields were finally computed on the basis of pounds of green shelled peas produced from a measured acre.

In addition to the above criteria grade information for the peas from each plot was obtained.

RESULTS

The results of the 1940 experiments are given in table 1 and are illustrated graphically in figures 1–4. From these data it can be seen that the stand was increased by seed treatment in every case. The percentage increases for the Spergon plots over the untreated plot being 23, 42, 41, 22, 11, and 1.2 per cent, an average of 23 per cent increase in stand. The increase obtained by the use of New Improved Ceresan also amounted to 23 per cent, while nitrogen inoculation increased the stand by 18.5 and 1.6 per cent, the average being 10 per cent. It was possible at all of the locations to pick out the nontreated plot by the uneven stand and the general prevalence of bare areas, usually scattered uniformly throughout the plot.

TABLE 1.—*Summary of field experiments with Spergon on pea seeds in Minnesota, 1940*

Location	Variety	Treatment	Date of planting	Date of harvest	Av. stand[a]	Av. length of plants (in.)[b]			Infection rating[c]	Yield per acre
						Crown–tip	Roots	Total		
										lb.
Plainview (Schultz)	Prince of Wales	Check	May 17	......	65.3	17.5	3.5	21.0	2.0	......
		Spergon	“	......	111.8	22.8	4.2	27.5	0.7	......
Plainview (Seefeldt)	Prince of Wales	Check	May 18	......	56.6	21.0	3.6	24.6	2.2	......
		Spergon	“	......	97.3	20.10	3.3	23.4	1.0	......
Blue Earth	Alaskan	Check	April 24	June 25	96.0	21.7	5.1	26.8	3.5	1960.0
		Spergon	“	“	106.7	24.4	4.7	29.1	1.9	2347.4
Spring Valley	Perfection	Check	May 2	July 9	79.0	17.5	2.1	19.6	2.6	2013.4
		N_2 inoc.	“	“	93.6	17.4	2.4	19.6	2.5	1221.9
		Spergon	“	“	111.1	17.2	3.0	20.7	1.8	2316.7
Dodge Center	Alaskan	Check	......	......	122.0	35.3	5.1	40.4	2.6	......
		N_2 inoc.	......	......	124.0	35.0	4.7	39.7	2.8	......
		Spergon and N_2 inoc.	......	......	120.0	39.0	5.2	44.3	2.1	......
Fairmont	Teton	Check	May 4	July 3	66.8	30.4	4.2	34.6	3.4	1930.9
		N. Imp Ceresan	“	“	82.4	32.5	3.8	36.3	1.8	2268.7
		Spergon	“	“	84.6	33.3	4.7	38.0	2.5	2730.0
Owatonna	Alaskan	Check	May 2	July 2	103.0	25.7	4.7	30.4	2.8	2303.8
		Spergon	“	“	126.7	27.7	4.1	31.8	1.6	2243.4

[a] Stand: Each figure represents the average of ten counts made at random throughout the field 2 weeks before harvesting.

[b] Length: Figures represent the average of 50 plants.

[c] Infection rating: Refers to the degree of root rot present on the plants. Arbitrary infection classes of 0, 1, 2, 3, 4, were established. 0 represents no infection; 4, severe infection resulting in death of the plants. Figures represent the averages for 50 plants.

In every locality where Spergon was used the stand was very uniform over the entire field.

There was apparently no significant difference in the height of plants from treated and nontreated seed, although in every case but one, the plants from the former were slightly taller than those from nontreated seed. It was noticeable at all of the locations that the use of Spergon appeared to invigorate slightly the resultant plants, for, in addition to a perceptible increase in the height of plants, there was, apparently, also a more vigorous and extensive development of the root system. The above results were not analyzed for their statistical significance.

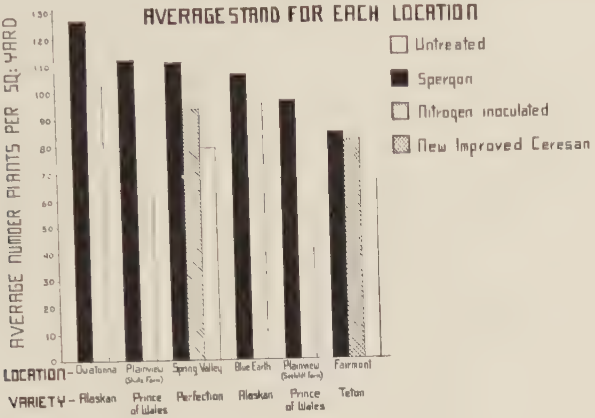

FIG. 1. Effect of seed treatment on the stand of peas.

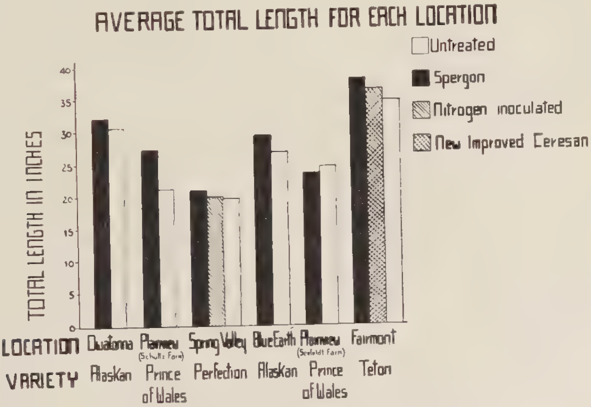

FIG. 2. Effect of seed treatment on the height of plants.

Using the scheme previously outlined, the severity of root-rot infection on the plants was determined in each plot at the different locations. The results given in table 1 are the averages of 50 determinations for each treatment, and from these results it is evident that seed treatment reduced the amount of root-rot development in every case. The percentage reduction in severity of root rot obtained by the use of Spergon, as compared with the untreated plots was respectively 43, 65, 31, 46, 55, and 26 per cent, the average being 44 per cent. It would seem to be significant that seed treatment in the case of the variety Alaskan, which has been bred for resistance to Rhizoctonia root rot, resulted in a reduction of 43 and 46 per cent in the amount of root rot present. New Improved Ceresan in the one location in which it was used reduced the amount of root rot by 47 per cent.

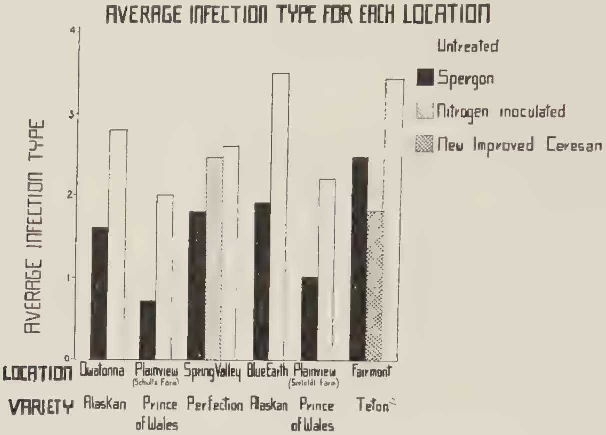

FIG. 3. Effect of seed treatment on the development of root rots.

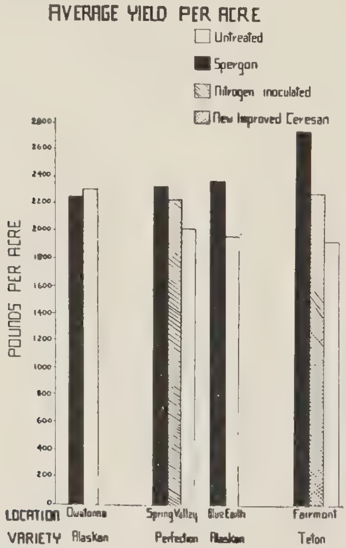

FIG. 4. Effect of seed treatment on the yield of peas.

The reduction in severity of root-rot infection obtained by the use of seed treatment is illustrated in figure 3.

The increases in yield obtained by the use of Spergon seed treatment, illustrated in figure 4, were, respectively, 303, 387, and 799 lb., while at

Owatonna a reduction of 60 lb. per acre was obtained. These figures in terms of percentage increase in yield as compared with the untreated plot may be expressed as an increase of 15, 20, and 41 per cent, with a reduction at Owatonna amounting to 2.16 per cent. Thus the average yield increase obtained by the use of Spergon amounted to 357 lb. of green, shelled peas to the acre, which is equivalent to an increase in production of 18 per cent over the untreated plot. New Improved Ceresan gave an increase of 338 lb. (17 per cent) as compared with 799 lb. (41 per cent) of green peas to the acre, obtained with Spergon at the same location.

Attempts also were made to determine whether seed treatment affected the quality of the crop grown from treated seed. In Minnesota the practice most commonly employed in the grading of canning peas is the use of the sample grader. In the grading process, employed at the canning factory, representative samples of the crop are passed over standard screens and the percentage of peas falling in the various sieve sizes determined, from which the field grade is computed by estimating the percentage weights of the peas falling in the desired sieve sizes. The number of sieve sizes used in determining the field grade varies with the variety of pea being tested; *i.e.*, in general the percentage weight of the 1, 2, and 3 sieve sizes would determine the field grade of the variety Alaskan, and the weight of sieve sizes 2, 3, 4, 5, and 6 would be used in determining the field grade of the large sweet varieties of peas. Thus, every packer is primarily interested in obtaining the greatest percentage possible of the desirable sieve sizes. In addition to the above method, a device known as the "tenderometer" is used by some packing plants to determine the quality of peas. This device measures the maximum force necessary to crush the sample. The reading obtained is an indication of the tenderness of the sample, that with the most desirable quality, having the lower reading. In this investigation, wherever possible, records were obtained on the quality of peas harvested from the various treated and nontreated plots. The records were obtained from the plant of the company concerned and the results are given in table 2. From these data it is apparent that seed treatment did not significantly affect the grade, although in the case of Alaskans a slight improvement is to be noted.

TABLE 2.—*Effect of seed treatment on pea quality as determined by sample grading and by tenderometer measurements*

Location	Variety	Treatment	Percentage of crop falling in each sieve							Tenderometer reading
			1	2	3	4	5	6	7 & 8	
Blue Earth	Alaskan	Nontreated	2	8	13	68	14	......	...	121
		Spergon	2	9	21	53	13	......	...	116
Fairmount	Teton	Nontreated	7	10	37	6	......	31	9	....
		N. Imp. Cer.	7	9	39	5	......	33	9	....
		Spergon	8	8	41	6	......	29	8	....

TABLE 3.—*Summary of field experiments with Spergon on Perfection peas at Spring Valley, Minnesota, 1941*

Treatment	Planting date	Date of harvest	Average length of plants in inches, June 5 and 6		Average length of plants in inches, June 25 and 26		Stand, June 5 and 6	Stand, June 25 and 26	Yield per acre in pounds
			Roots	Total	Roots	Total			
No treatment	April 30	July 6	3.3	14.3	6.8	27.4	91	86	2560.4
Spergon	May 2	July 8	3.9	17.3	7.3	28.7	107	87	2915.2
Spergon plus inoculation	May 1	July 7	3.2	15.5	7.2	31.5	105	93	2650.8

TABLE 4.—*Significance*[a] *of data on stand and plant length given in table 3*

Date taken	Comparison	Root length	Total vine length	Stand
June 5 and 6	Spergon—control	Highly significant[b]	Highly significant	Significant[c]
June 5 and 6	Spergon plus inoculation—control	Not significant	Significant	Significant
June 5 and 6	Spergon plus inoculation—Spergon	Highly significant	Highly significant	Not significant
June 25 and 26	Spergon—control	Not significant	Significant	Not significant
June 25 and 26	Spergon plus inoculation—control	Not significant	Highly significant	Not significant
June 25 and 26	Spergon plus inoculation—Spergon	Not significant	Highly significant	Not significant

[a] Significance was determined by comparing the means and using the standard error of the difference.
[b] Significance at 1 per cent level.
[c] Significance at 5 per cent level.

With the same variety, also, tenderometer readings indicated that the peas from Spergon-treated seed were of slightly better quality. The results, however, are not significant and it was concluded that seed treatment in these experiments neither increased nor lowered the quality of the crop.

In 1941 the size of experimental plots was increased to 10 acres, each, and all of the work was done in cooperation with the Reid Murdoch Canning Company on the Pattridge Farm at Spring Valley, Minnesota. All of the plots were planted to the variety Perfection at a seeding rate of 4.25 bu. per acre, and the seed was treated with Spergon applied at the rate of 2 oz. to the bushel. The results obtained in 1941 are given in table 3 and the statistical significance of these data is given in table 4.

From the records taken on June 5 and 6, one month after planting, and one month before harvest, it is apparent that a significant increase in stand resulted from the use of Spergon. The differences in root length and total vine length also were highly significant. A further significant increase in stand and length of vine was obtained when nitrogen inoculation was combined with the seed treatment. On the other hand, when similar records were taken on June 25 and 26, approximately 8 weeks after planting and 2 weeks before harvest, there was no significant difference in stand obtained by seed treatment. The differences in root length were not significant, but a significant difference in total vine length was again obtained, the difference being greater when nitrogen inoculation was combined with Spergon treatment.

An increase in yield of 13.9 per cent was obtained over nontreated seed by the use of Spergon, but no increase in yield was obtained when this treatment was combined with nitrogen inoculation. This failure of nitrogen inoculation plus Spergon to result in an increased yield is difficult to explain, although a marked decrease in yield as compared with nontreated seed resulted during the previous season, where nitrogen inoculation was used. Observations at that time indicated that the bacterial gel used for nitrogen-inoculation purposes may serve as a pabulum for root rotting fungi, increasing the injury that they cause. The results obtained in 1941 would indicate that seed treatment with Spergon is effective in combating pre-emergence damping off, but was not so effective in preventing root rot or post-emergence damping-off. The initial stimulus apparently was sufficient to result in an increase in yield. The same situation did not exist during the previous season as marked increases in stand were obtained at harvest time. Environmental conditions, however, were very different, 1940 being relatively dry until harvest time, whereas, in 1941, very wet conditions prevailed for several weeks before and until harvest time.

CONCLUSIONS

The increases in yield obtained by seed treatment in itself would seem to indicate that root rots of peas constitute a hazard to the pea industry in Minnesota, and during the course of this investigation, at least, must have considerably reduced the yields. The prevalence of root rots is not generally recognized by the canners, and the losses resulting from such diseases are not at present realized, possibly because there has been no adequate standard of comparison, which experimental seed treatment experiments furnish, from which such losses could be computed. Isolations made from root-rot-infected plants in 1940 indicated that 3 fungi were involved as the cause of pea root rots in Minnesota. *Rhizoctonia* sp., *Fusarium martii* var. *pisi,* and *Aphanomyces euteiches* were isolated from diseased peas from different locations during 1940. In 1941 a species of *Pythium* was isolated from the diseased plants. The results obtained in this investigation indicate that Spergon was the most satisfactory seed protectant tested for canning peas in Minnesota, although it was not so effective in preventing the development of root rots late in the season, which also is true of other proprietary seed protectants. The apparent stimulation to seed treated with this material, as expressed by increased rate of emergence, greater total vine length and more vigorous root development, would seem to be sufficient to be responsible for increased yields when conditions suitable for the development of root rots prevail.

SUMMARY

Root rots of peas were present in epidemic proportions in the locations in which the above investigations were conducted during 1940 and 1941.

Seed treatment with Spergon in 1940 resulted in an average increase in stand of plants amounting to 23 per cent, New Improved Ceresan in one case gave the same increase, while nitrogen inoculation gave an increase in stand of 10 per cent over the untreated plot.

The height of plants was slightly increased in all cases but one by seed treatment in 1940, but the increases did not appear to be significant. The increases in total vine length obtained in 1941 were statistically significant. Spergon appeared to materially stimulate the root development of plants from seeds treated with this material.

The incidence of root rot was greatly reduced by seed treatment.

Increases in yield from 300 to 800 pounds of green, shelled peas to the acre were obtained at three locations by treating seed with Spergon, the average increase amounting to 357 pounds or 18 per cent over untreated seed in 1940.

Results obtained in 1941 substantiated the findings of 1940.

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THE ADHERENCE OF FIXED COPPER FUNGICIDES AS INFLUENCED BY SPRAY SUPPLEMENTS

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Supplementary materials are frequently added to spray mixtures of the fixed copper fungicides to improve such physical performance of the spray as coverage and adherence. The choice of materials used as spreaders and stickers has, in practice, been made largely by trial and error, and very little fundamental research has been reported on the performance of these substances. The purpose of this investigation was to study the effect of the commonly available supplementary materials upon the properties of copper-fungicide spray deposits in order to facilitate the selection of the most suitable materials.

MATERIALS

In order that the fungicidal efficiency of the spray be maintained, it is desirable that the chemical properties of the fungicide be not adversely affected by the addition of the supplementary materials. In other words, the supplements should be compatible with the fungicide.

A large number of supplements are being used commercially to adjust the physical properties of copper fungicide sprays (3). These materials may be classified according to their function as follows:

(a) Materials that act physically to spread the spray liquid, *e.g.*, sulphonated oils and sulphite waste liquor products.

(b) Materials that act physically to stick the spray deposit, *e.g.*, resins and mineral oils.

(c) Materials that act physically as spreaders and form gelatinous precipitates, which function as stickers, *e.g.*, soya flour, skim milk, etc.

During the last few years, materials of vegetable and animal origin, such as flours and casein materials, have been extensively used as spray adjuvants by experiment station research workers, as well as by commercial growers. These supplementary materials have been of particular value in spray mixtures, since they tend to impart a gelatinous character to the spray residue of fixed copper fungicide, and to improve its adherence (2). For this reason, materials possessing these properties were selected for this study. It will be noted from the analysis of these materials that they contain various amounts of proteins:

COMPOSITION OF SUPPLEMENTS INCLUDED IN ADHERENCE TESTS

Wheat flour—patent flour milled from southern wheat
(Analysis by General Mills laboratory)

	Per cent
Protein	8.600
Ash	.765
Moisture	13.5
Fat	.4

High protein wheat flour	
Protein	17.5
Soya flour—(Analysis by Central Soya Co. laboratory)	
Protein	53.5
Carbohydrates	30.0
Starch	Nil
Fat	.3
Ash	5.7
Moisture	7.0
Calcium caseinate	
Protein	42.0
(Calcium caseinate is a reaction product of casein and hydrated lime, which contains an excess of free lime.)	
Skim milk	
Protein	36.0

Since the basic sulphates of copper are most widely used for fungicidal purposes, a basic copper sulphate containing 26 per cent copper as metallic was selected for these studies.

LABORATORY TESTS

In order to investigate more completely the effect of supplements on copper-fungicide sprays, studies were conducted on the adherence of the spray deposit, the solubility of the copper fungicide in the spray mixture and the effect of soluble copper on the performance of the spray supplement.

Adherence Tests

The method of determining the adherence of the copper-fungicide spray deposit was patterned after that of Young and Beckenbach (6). The equipment used consisted of a DeVilbiss atomizer mounted in a permanent position over a container equipped with an agitator. The glass slides (10″ × 10″) were supported at an angle of 45° at a distance of 30″ from the atomizer tip. A removable baffle imposed between the atomizer and the plate permitted deposition of the spray for definite intervals, and a uniform amount of material was deposited on replicate plates. The interval of deposition for each spray mixture was adjusted to the point of incipient running of the spray deposit in order to determine the maximum deposit obtainable. Quadruplicate slides were prepared for each spray mixture. The deposit on 2 plates was removed by washing with dilute sulphuric acid and analyzed for copper. The 2 remaining slides were dried at room temperature for 24 hours and then subjected to washing by artificial rain for 30 minutes. The volume of water running off from each plate was approximately 1000 ml. The residue was removed from the washed plates and analyzed for copper, as above.

Solubility Tests

The following method was used for determining the amount of soluble copper. A suspension of the copper fungicide and supplement was agitated for 16 hours at 76° F. in 3000 ml. of water, and allowed to settle for 24 hours. An aliquot portion of the clear supernatant liquid was withdrawn and analyzed for soluble copper.

Sorption Tests

The sorption of soluble copper by the supplements was determined by treating a suspension of the supplement with a known quantity of soluble copper at a definite concentration, allowing to stand for 24 hours, and analyzing an aliquot portion of the clear supernatant liquid for soluble copper. The difference between the initial and final copper content in solution represented the quantity of copper sorbed by the supplement.

The laboratory adherence tests were conducted with mixtures the composition of which were similar to those used in the field, *i.e.*, they contained the copper fungicide, supplement, and hydrated lime. However, in the solubility and sorption studies, lime was omitted from the systems in order to determine the effect of the supplements on the solubility of the copper fungicide and sorption of soluble copper.

LABORATORY RESULTS AND OBSERVATIONS

Adherence Studies

The results secured from the laboratory adherence tests of sprays containing the various supplements are summarized in table 1, and shown graphically in figure 1. The greatest average initial deposit of copper at the point of incipient running of the spray deposit was obtained with soya flour followed in decreasing order by wheat flour, calcium caseinate, skim milk, and albumin. The greatest average copper residue, after subjecting the dried deposits to artificial rainfall, was obtained with soya flour, fol-

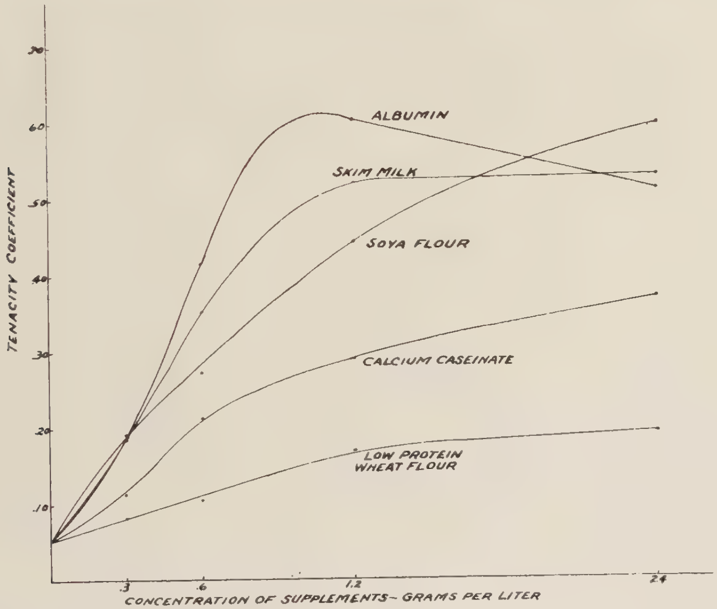

FIG. 1. Effect of spray supplements on the adherence of basic copper sulphate, laboratory tests.

TABLE 1.—*Effect of spray supplements on the adherence of basic copper sulphate. Summary of the results expressed as the average of the laboratory and 1939 and 1940 field spray tests*

Supplement[b]	Laboratory			Orchard 1939			Orchard 1940		
	Spray deposit Milligrams copper per 100 sq. in.		Tenacity coefficient[a]	Spray deposit Milligrams copper per 100 sq. in.		Tenacity coefficient	Spray deposit Milligrams copper per 100 sq. in.		Tenacity coefficient
	Initial	Residual		Initial (Cumulative)	Residual		Initial (Cumulative)	Residual	
Low-oil soya flour	13.25	5.32	.401	10.21	6.82	.668	20.89	14.58	.697
Low-protein wheat flour	11.30	1.54	.136	8.67	4.75	.548	11.34	6.91	.609
Skim milk	10.37	4.25	.410	10.39	8.52	.820	12.84	7.58	.590
Calcium caseinate	10.76	2.69	.250	7.91	4.35	.550	14.39	8.43	.586
Albumin	7.52	3.37	.448	9.81	6.19	.631	10.00	5.64	.564
None	7.97	.49	.051	6.66	3.13	.470	11.04	6.20	.561

[a] Tenacity coefficient = Ratio $\frac{\text{Residual spray deposit}}{\text{Initial spray deposit}}$.

[b] Dolomitic lime included in all sprays. Note:—Rainfall 1939 14.60 inches.
“ 1940 16.10 “

Spray formula for 1939 and 1940 Orchard tests:—2.5 lb. Basic copper sulphate; 2.5 lb. lime; 100 gal. water.

The difference between the tenacity values of the laboratory and the field tests is undoubtedly due to the difference in the surface of the glass plates and of the apple foliage. The character of the surface has as much influence on adherence as the spray material itself.

lowed by skim milk, albumin, calcium caseinate, and wheat flour. From these data, the tenacity coefficient of the spray deposit (5) was calculated. This coefficient expresses the ratio of the residual deposit to the initial spray deposit. The sprays containing albumin as the supplement had the highest average coefficient of tenacity, followed in decreasing order by those containing skim milk, soya flour, calcium caseinate, and wheat flour.

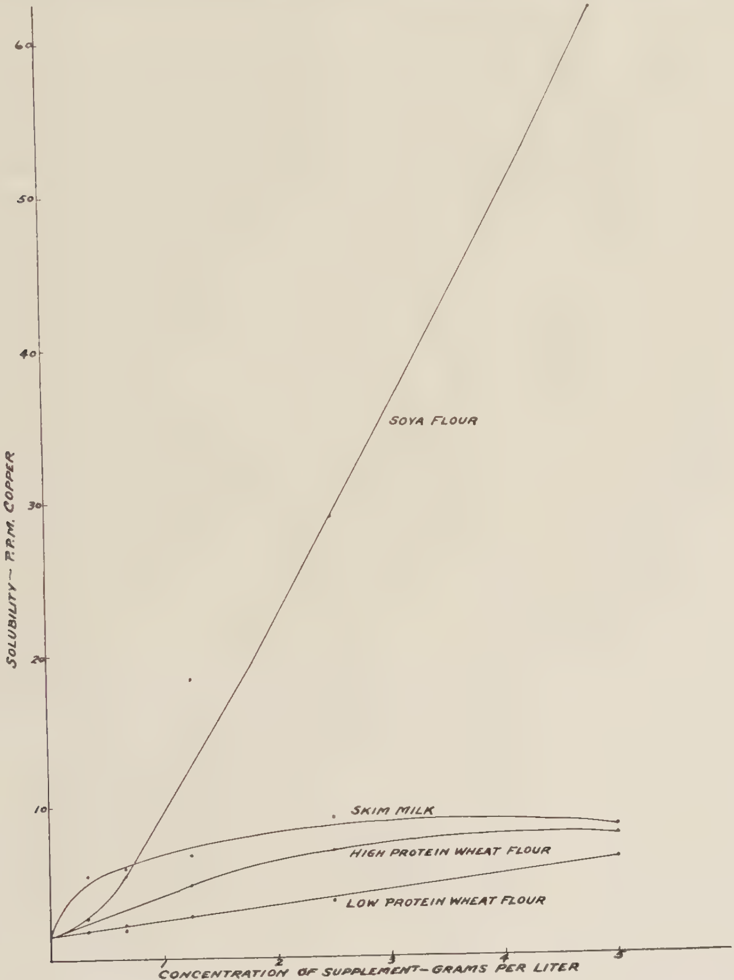

FIG. 2. Effect of spray supplements on the solubility of basic copper sulphate, laboratory tests.

It is of interest to note that, although the sprays containing albumin had the highest coefficient of tenacity, the residual deposit for the albumin mixture was 37 per cent less than that for soya flour. The increased residual deposit which may be secured with supplementary materials is particularly important from the standpoint of the sufficiency of the coverage. Since the magnitude of the residue is a function of the initial spray deposit, the supplementary materials which can be used to build up the initial deposit of copper are of particular value. From the above it is obvious that effective supplements, such as materials containing protein, may be used advantageously to build up the spray residue.

Solubility Studies

The loss of copper during rainfall may result from leaching of soluble copper, as well as from mechanical washing away of the solids from the spray residue. Although a primary object of using spray supplements is to secure improved adherence of the spray residue, the solubility of the copper fungicide should not be reduced by the supplements. It is apparent that the dissolving action of the spray supplement upon the fixed copper fungicide plays a very important role in the effectiveness of the spray, since it causes an increase in the concentration of available copper ion. The effect of this dissolving action upon the retention of copper is of particular interest. In this connection, a study was made on the effect of spray supplements upon the release of soluble copper from the copper fungicide.

TABLE 2.—*Effect of spray supplements on the solubility of basic copper sulphate, and the retention of soluble copper by supplements. Laboratory tests*

Supplements	Soluble copper in copper fungicide supplement suspensions p.p.m. copper	Soluble copper sorbed by supplements mg. Cu/gm supplement	Analysis of sorption complex[a]		Adherence of sorption complex		
			Copper content	Water-soluble copper of total copper	Initial deposit mg. Cu per 100 sq. in.	Residual deposit mg. Cu per 100 sq. in.	Tenacity coefficient
		Per cent	*Per cent*				
Calcium caseinate	103.6	1300	2.62	2.0	....	...	....
Low-oil soya flour	24.5	2050	1.76	14.3	2.4	.6	.250
Powdered skim milk	7.2	762	1.86	25.3	....	...	....
High-protein wheat flour	5.4	810	0.58	27.6	....	...	....
Low-protein wheat flour	3.4	190	0.11	36.4	.88	.2	.227
None (check)	1.5	....	....	....	....	...	....

[a] The term "Sorption Complex" represents the supplement containing sorbed copper.

Column 1—Determined by analysis from the suspension of basic copper sulphate and supplement in water.

Column 2—Represents the difference between the amount of copper in initial solution and final solution.

Column 3—Refers to the washed and dried residue from treatment of supplement with soluble copper.

Column 4—Determined from the suspension of residue (Column 3) in water.

The difference between the amount of soluble copper sorbed by the supplements (Column 2) and the copper content of the sorption complex (Column 3) is due to the fact that the supplements themselves are partially soluble in water.

The data from the solubility tests are shown graphically in figure 2, and are summarized in table 2. It was found that the highest concentration of soluble copper was obtained in sprays containing calcium caseinate as the supplement (103.6 p.p.m. soluble copper) followed in decreasing order by soya flour (24.5 p.p.m.), powdered skim milk (7.2 p.p.m.), high-protein wheat flour (5.4 p.p.m.), low-protein wheat flour (3.4 p.p.m.) and the check, which contained no supplement (1.5 p.p.m.).

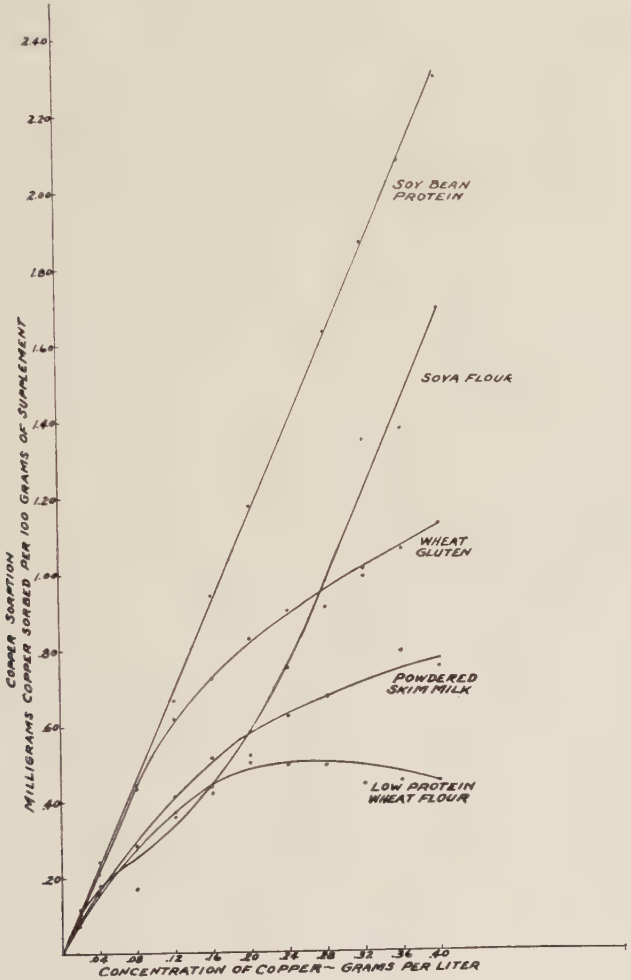

FIG. 3. Sorption of copper by spray supplements.

It was noted that soya flour, which caused a higher solubility of the copper fungicide than wheat flour, imparted a greater tenacity to the copper residue. From this it appears that, during rainfall, soya flour must prevent a rapid dissipation of the soluble copper.

Sorption Studies

A study on the performance of the protein-containing supplements in the presence of soluble copper showed that the materials tend to absorb copper ions from the solution, and return them in the form of a complex. The extent of sorption of soluble copper by the supplements was determined by measuring the amount of copper in solution before and after treating the supplements with copper sulphate solution. It is apparent from the curves (Fig. 3) that the amount of copper sorbed by each supplement was dependent on the concentration of soluble copper until the sorption capacity of the supplement was reached. Further increase in the concentration of soluble copper did not cause an additional amount of copper to be sorbed.

A further study showed that the sorption capacity of soya protein was greater than that of soya flour, while in the case of wheat gluten and wheat flour an even greater relative increase in the sorption capacity was secured. This indicates that it is protein that forms the sorption complex with soluble copper. In other words, the retention of soluble copper apparently is caused by the formation of a copper-protein sorption complex. The sorption capacity of soya flour was found to be approximately 3 times that of wheat flour. Since the protein content of soya flour is also about 3 times that of wheat flour, it appears that the sorption capacity of the supplement is largely dependent upon its protein content. However, it is possible that the source of the protein, as well as its treatment during the manufacturing process, may influence the performance of these materials.

In the sorption tests, the saturated residues, which contained the sorption complexes formed by the various supplements, were filtered and washed to remove free copper sulphate. A portion of the filter cake was dried and analyzed for copper (Table 2). The availability of the copper in the resultant cake was determined by solubility tests.

Adherence tests were conducted on a portion of the undried filter cake that was redispersed in water. It was found that the tenacity coefficient of the soya flour sorption complex was 0.250, while that of the wheat flour sorption complex was 0.227. However, the magnitude of the deposit of the soya-flour complex was approximately 3 times that of the wheat-flour complex.

Due to the sorption capacity of the supplement for soluble copper, the amount of copper dissolved by the supplement must include both the free soluble copper and the copper sorbed. In other words, the solubility of the copper fungicide in suspension with the spray supplements (Table 2) represents the difference between the total dissolved copper and the amount of soluble copper sorbed by the supplement. In the copper fungicide-supplement suspension there apparently takes place: (1) solution of the copper fungicide; and (2) subsequent sorption of the soluble copper by the supplement until an equilibrium is reached. It should be noted that the amount of soluble copper, as determined at the point of equilibrium, is of particular value, since it furnishes information on the amount of available copper for the control of fungus disease on the one side, and on the safety of the spray on the other.

In practice, once the spray residue has become dry, the copper content of the copper-protein complex and its solubility are factors in the availability of copper in the spray residue, as well as in the loss of copper by leaching. In this connection, the soya-flour complex has a higher copper content (1.76% Cu) than the wheat-flour complex (0.11% Cu), while the percentage of soluble copper in the soya-flour complex (14.3%) is less than in the wheat-flour complex (36.4%). The total amount of soluble copper from the soya-flour complex (2.5 mg. per gram) is, however, approximately 6 times higher than the soluble copper from the wheat-flour complex (0.4 mg. per g.). On the basis of the amount of soluble copper, it seems that the effectiveness of the soya-flour complex would be greater than that of the wheat-flour complex. Soya flour, due to its greater capacity for the sorption of soluble copper, may considerably reduce the loss by rainfall of copper released from the copper fungicide.

Field Tests

To determine the effect of the various supplements on the adherence of basic copper sulphate on the foliage, field tests were conducted at the Wilson apple orchard, Epworth, Ga. Three replicate plots, containing an average of 8 trees each, were laid out for each test. Six copper cover sprays were applied beginning 2 weeks after the petal fall application of lime-sulphur.

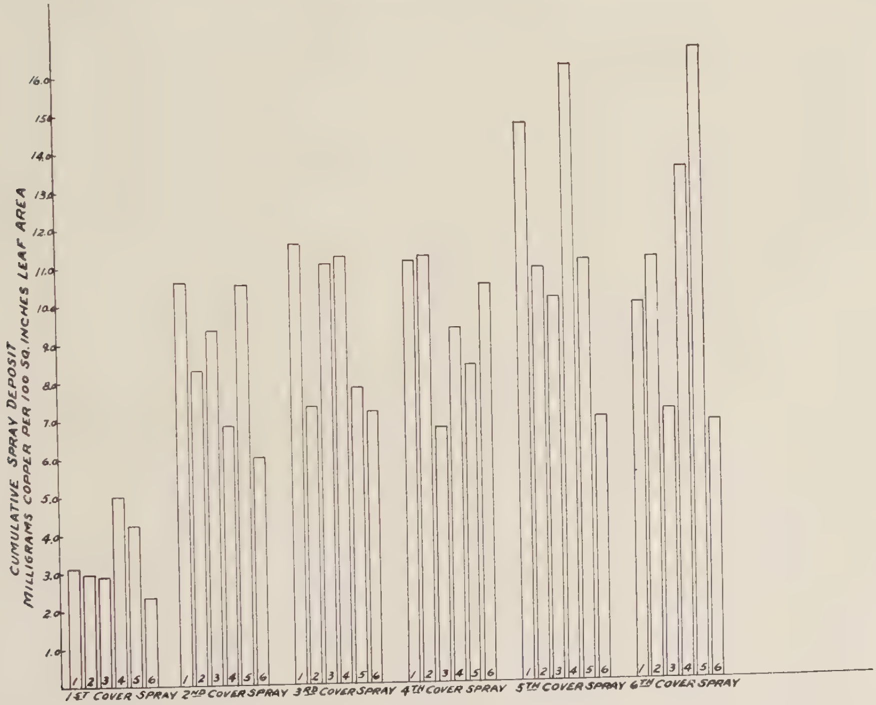

FIG. 4. Effect of spray supplements on the deposit of basic copper sulphate on apple foliage, 1939. 1, soya flour; 2, wheat flour; 3, calcium caseinate; 4, skim milk; 5, albumin; 6, check (no supplement).

In order to compare the effect of the various supplements on the deposit and adherence of the copper fungicide, leaf samples were collected from each series of plots (4) and their spray residue analyzed quantitatively for copper (1). Care was taken to get representative samples of leaves from each plot and to prevent any loss of spray residue during subsequent treatment. Samples were collected directly after each spray application to determine the magnitude of the deposit. In 1939, samples were collected after an interval of weathering, to determine the tenacity of the spray deposit. In 1940, it was possible to collect samples at approximately weekly intervals, as well as immediately before and after each spray application. It was possible, in this manner to obtain a comprehensive comparison of the magnitude and tenacity of the spray deposit.

FIELD RESULTS AND OBSERVATIONS

The data from the orchard adherence tests are summarized in table 1 and illustrated in figures 4 and 5.

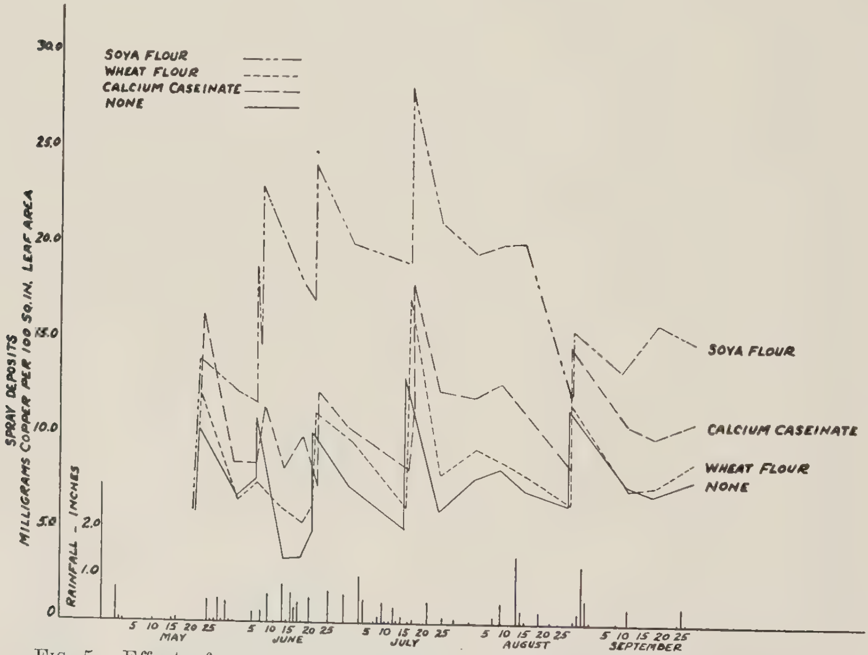

FIG. 5. Effect of spray supplements on the adherence of basic copper sulphate to apple foliage. 1940.

In the 1939 tests, the largest average deposit was obtained with skim milk, followed in decreasing order by soya flour, albumin, wheat flour, calcium caseinate and the check (no supplement). The largest average residue was obtained with skim milk, followed in order by soya flour, albumin, wheat flour, calcium caseinate and the check. The highest coefficient of tenacity was shown by sprays containing skim milk, followed in order by soya flour, albumin, calcium caseinate, wheat flour and the check.

In the 1940 tests, the largest average deposit was obtained with soya flour, followed in decreasing order by calcium caseinate, skim milk, wheat flour, check and albumin. The largest average residue was obtained with soya flour, followed in order by calcium caseinate, skim milk, wheat flour, check and albumin. The soya flour spray deposit had the highest coefficient of tenacity in 1940, followed by wheat flour, skim milk, calcium caseinate, albumin and the check.

The order of the comparative performance of the supplements in 1940 was somewhat different from their order in 1939. This can be attributed to the difference between the weather conditions and the amounts of rainfall during the two seasons.

The results obtained in the 1940 field tests show that there was a substantial difference between the magnitude of the spray residue obtained with soya flour and wheat flour. It should be emphasized that the magnitude of the residual spray deposit is just as important from the standpoint of sufficient coverage as the coefficient of tenacity of the residue. As an example, the tenacity coefficient of the 1940 sprays containing calcium caseinate was lower than that of sprays containing wheat flour, yet the amount of residual spray deposit was greater with calcium caseinate.

DISCUSSION

A study of laboratory and field results indicates that the spray supplements of the type included in these tests, function not only as spreaders and stickers for the spray deposit, but also as activators for the fixed copper fungicides. The supplements, when included in spray mixtures, exert a distinct dissolving action on the fixed copper fungicide, forming a sorption complex with the dissolved copper. This complex is an available source of copper in addition to the small quantity (1.5 p.p.m.) that is in solution.

The degree of activation of the basic copper sulphate appears to depend upon the protein content of the supplement. The effect of the protein content of the supplement can readily be seen from the superior performance of soya flour containing 53.5 per cent protein as compared with low protein wheat flour containing 8.6 per cent protein. These two flours are representative of high and low protein supplements, respectively.

The above indicates that a certain correlation exists between the dissolving action of the supplement upon the copper fungicide and the sorption of soluble copper by the supplement on the one hand, and the subsequent liberation of copper ion from the sorption complex on the other.

The sorption complex formed by the action of the supplement upon the fixed copper fungicide, plays a very important role in the adherence of the spray residue. The improved adherence obtained by the use of materials of high protein content as supplements, such as soya flour, can be partly attributed to the formation of this gelatinous sorption complex. The inferior adherence obtained with wheat flour probably is due to its low protein content and to the low amount of the sorption complex formed by this supplement. The supplements that have a high sorption capacity, such as soya flour, increase the tenacity of the spray deposit to a greater extent than supplements of low sorption capacities, such as wheat flour. Apparently, a correlation exists between the sorption capacity of the supplements for soluble copper and the magnitude and tenacity of their respective spray residues.

From the above it seems that the improved adherence and activation of basic copper sulphate by the action of protein-containing supplementary materials may be readily utilized for the improved performance of fixed copper fungicides. Since the action of the supplements depends to a large extent on the proteins and their sources, it seems that the vegetable materials, such as soya bean protein, may be used advantageously to improve the performance of basic copper sulphate sprays, both from the standpoint of adhesiveness of the spray residue and from the activation of the copper fungicide.

SUMMARY

The performance of protein-containing spray supplements were studied from the standpoint of their effect upon the adherence and activation of fixed copper fungicides.

The results secured from spray tests conducted in the field and laboratory showed that:

The magnitude and tenacity of the spray residue are considerably improved by supplements containing protein.

Supplements, according to their protein content, exert a dissolving action on the fixed copper fungicide. The soluble copper released from the fixed copper fungicide is sorbed by the protein to form a gelatinous sorption complex, which increases the adhesiveness of the spray deposit.

A certain correlation exists between the dissolving action of the supplement upon the copper fungicide, and the subsequent liberation of soluble copper from the sorption complex.

A certain correlation exists between the sorption capacities of the supplements for soluble copper and the magnitude and tenacity of their respective spray residues.

COPPERHILL, TENNESSEE.

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POTATO-SCAB GARDENS IN THE UNITED STATES

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It has been known for some time that there are a comparatively large number of physiologic forms of *Actinomyces scabies* (Thaxter) Güssow, the organism that causes common scab in the potato. It has been observed, too, that the intensity of scab epidemics varies from year to year and from place to place, because of climatic and soil conditions. In any effort to breed scab-resistant varieties or to determine the genetic behavior of reactions to scab, physiologic forms and environmental factors must be given consideration. Varieties resistant to scab in one section of the country under a particular set of environmental conditions may be susceptible in another section. To determine to what extent a breeding program is affected by these sources of variation the cooperators in the National Potato Breeding Program in a conference at Ithaca, N. Y., in 1937, suggested that a number of uniform scab gardens be established in various sections of the country. Six such gardens were established in 1938—Elk River, Minn., Greeley, Colo., Lake City, Mich.,

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Ithaca, N. Y., Kingston, R. I., and Presque Isle, Maine. In 1939 the number of gardens was increased by additional ones at Avon, Colo., Grand Rapids, Minn., Clear Lake, Iowa, Madison, Wisc., Manhattan, Kan., Baton Rouge, La., and La Fayette, Ind. In 1940 Shafter, Calif., Wooster, Ohio, Logan, Utah, and Laramie, Wyo., were added to the list.

MATERIALS AND METHODS

As a preliminary step in establishing these gardens it was suggested to the collaborators in the various State experiment stations where potato-breeding programs are in operation, that they send seed stocks of potato varieties they wished tested. Three varieties were received from F. A. Krantz of the Minnesota Agricultural Experiment Station and 11 from Donald Reddick of the New York Agricultural Experiment Station at Cornell University. In addition, 91 varieties that had shown various reactions to scab in the tests at Presque Isle, Maine, for the previous 2 years were assembled at Beltsville, Md., and distributed to the various stations.

Due to the small amount of seed stock available, only enough for duplicate rows of 5 hills of each could be sent to each station. This stock was planted in 1938 in rows adjacent to a row of a susceptible check variety. Scab data were secured by the various collaborators on each replicate and on its corresponding check. From the results obtained it was apparent that in addition to physiologic forms and environmental factors the individuality of the various persons who took the data was a source of variation. To eliminate this last factor as much as possible, the scabbiest tuber from each hill in the test with its corresponding check was sent to Beltsville, Md., in 1939, where the scab readings were all made by one person. In 1940 the data for the gardens in the eastern section of the United States were secured by one man, those for the Northwestern and Plateau States by another. These men had previously worked together so that the readings for that year are considered comparable. The method of taking the scab data in 1939 and in 1940 was similar to that described by Clark *et al.*[6] Two criteria were considered in judging the reactions of varieties and checks, *i.e.*, the type of scab pustule and the percentage of tuber surface covered with pustules.

Types of scab pustules were recorded in three categories: 1. Pustules relatively large and deep. 2. Pustules relatively large but superficial. 3. Pustules small and superficial.

The percentages of tuber surface covered with pustules were recorded in six classes:

Class	Extremes	Mean
	Per cent	*Per cent*
Trace	0–1	0.5
1	1–20	10.0
2	21–40	30.0
3	41–60	50.0
4	61–80	70.0
5	81–100	90.0

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[6] Clark, C. F., F. J. Stevenson, and L. A. Schaal. The inheritance of scab resistance in certain crosses and selfed lines of potatoes. Phytopath. **28**: 878–890. 1938.

RESULTS

As the data for 1938 were obtained by a number of methods, they might be considered reliable in making the comparisons between varieties for a particular garden but not between varieties for all gardens. As the chief objective of the tests was to compare the behavior of the varieties in a series of gardens, the 1938 data are not used in this paper. The records for 1939 and 1940, on the other hand, were all secured by uniform procedure, and should be reliable for making comparisons within and between tests.

If the checks for both replications of a particular variety did not show 50 per cent of the tuber covered with No. 1 pustule type of scab, the data were not used in the final analyses. This was done to avoid pseudo-resistance. The standard is admittedly arbitrary and rather drastic, but if the readings on the varieties under test are to be considered dependable the checks must show the prevalence of a heavy epidemic.

The variation in scab incidence within gardens, between gardens, and from year to year, as determined by the susceptible check, greatly reduced the number of data that could be considered uniform. Twenty-two named and numbered varieties met the prescribed limits in both replications in 4 gardens and for 2 years. The data for these were used in the analyses of the variances of types of pustule, of percentages of tuber surface covered, and of the covariances between these two criteria.

PUSTULE TYPES

Type of pustule is considered by most investigators to be the more important criterion for judging the scab resistance of a potato variety. If large, deep pustules are found on the surface of the tubers the variety is considered susceptible, regardless of the percentage of tuber surface covered with the disease. The data for pustule types are given in table 1.

The checks all produced the No. 1 type of scab and are not recorded in the table. It is evident that some varieties are quite consistent in the character of scab pustule produced. Houma, the most susceptible variety in the list, showed the No. 1 type in all but one test. The exception was at Greeley, Colo., in 1939, where a No. 2 type of pustule was produced. Sebago showed a small degree of resistance with 10 records of No. 2 type and 6 of No. 1. It will be noted that in 2 tests of this variety one replication read No. 1, the other No. 2. Hindenburg with one exception produced No. 3 type. In Michigan in 1939 it produced No. 2 type. The reaction of Richter's Jubel was quite consistent with the No. 3 type in all except the 1940 Michigan test. In that test one replication read No. 1, the other No. 2. It is very unusual to find the No. 1 type on this variety. Arnica showed somewhat less resistance than either Hindenburg or Richter's Jubel, but a much higher degree of resistance than Houma, Sebago, or the susceptible checks. Certain of the unnamed varieties, such as AAO–9, 528–14, 528–119, 627–8, 627–213, and 1037–5, showed a relatively high degree of variation in the types of scab produced. If Hindenburg is taken as the standard for resistance, and twice the standard error of a difference between means as the criterion of significance, then Richter's Jubel, 245–36, 256–11, 528–242, 627–7, and 627–164 are in the same class as Hindenburg so far as the type of pustule produced on the tubers in this series of tests is concerned. It should be noted that two of the resistant varieties had Richter's Jubel as a parent, one had Arnica, and two had Hindenburg.

TABLE 1.—*Data for type of scab pustules on 22 potato varieties grown in duplicate rows (A and B) in four gardens in 1939 and 1940*

Varieties	Presque Isle, Maine				Greeley, Colo.				Elk River, Minn.				Michigan				Mean of 16 tests
	1939		1940		1939		1940		1939		1940		1939		1940		
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
																	Per cent
Sebago	2[a]	2	1	1	2	2	2	2	1	2	2	2	2	1	1	1	1.6
Hindenburg	3	3	3	3	3	3	3	3	3	3	3	3	2	2	3	3	2.9
Richter's Jubel	3	3	3	3	3	3	3	3	3	3	3	2	3	3	1	2	2.8
Arnica	3	2	2	3	2	2	3	3	3	3	3	3	3	3	2	2	2.6
Houma	1	1	1	1	2	2	1	1	1	1	1	1	1	1	1	1	1.1
AA0–9	1	2	1	1	3	3	3	3	2	2	2	2	2	1	1	1	1.9
245–36[b]	2	3	2	2	3	3	3	3	3	3	3	3	3	3	2	2	2.7
256–11	3	3	2	2	3	3	3	3	3	3	3	3	3	3	2	2	2.8
528–14	2	3	1	1	2	2	3	3	3	3	2	2	3	3	1	2	2.3
528–119	2	2	1	1	3	3	3	3	2	2	3	3	2	2	1	1	2.1
528–242	2	3	3	3	3	3	3	3	3	3	3	3	2	3	2	2	2.8
574–20	3	3	2	1	2	2	3	3	2	2	3	2	2	2	1	2	2.2
627–7	3	3	2	2	3	3	3	3	3	2	3	3	3	3	2	2	2.7
627–8	3	2	1	1	2	2	3	3	3	3	2	2	1	2	1	2	2.1
627–18	2	3	1	2	3	3	3	3	2	3	2	2	3	3	2	2	2.4
627–103	3	3	2	2	2	2	3	3	2	3	3	3	3	3	1	1	2.4
627–126	2	2	1	1	2	2	3	3	3	2	3	3	3	3	2	2	2.3
627–164	2	3	2	2	3	3	3	3	3	3	3	3	3	3	2	2	2.7
627–210	3	2	2	1	3	3	3	2	2	2	2	3	3	3	2	2	2.4
627–213	2	2	2	3	3	3	2	2	2	2	2	2	1	3	1	1	2.1
627–273	2	2	2	2	3	3	3	3	3	2	2	3	2	2	1	2	2.3
1037–5	2	2	1	2	3	3	1	1	1	1	1	1	1	1	1	1	1.4
Difference for significance at 5-per cent level																	0.2

[a] Types of pustule:
1 = Relatively large and deep.
2 = " " but superficial.
3 = " small and superficial.

[b] Parents of the crosses represented in the table are:
245, 444–12 × Richter's Jubel
256, 444–12 × Arnica
528, Richter's Jubel × 44537
574, Arnica × 44537
627, Hindenburg × Katahdin
1037, 45208 × 44537

PERCENTAGES OF TUBER SURFACE COVERED WITH SCAB

The data for percentage of tuber surface covered with scab pustules are given in table 2. The susceptible checks were not included in the table, since the tubers of these were all heavily infested—none of them showing less than 50 per cent of their surface covered with scab.

TABLE 2.—*Percentage of tuber surface covered with scab pustules on 22 potato varieties grown in duplicate rows (A and B) in four gardens in 1939 and 1940*[a]

Varieties	Presque Isle, Maine				Greeley, Colo.				Elk River, Minn.				Michigan				Mean of 16 tests
	1939		1940		1939		1940		1939		1940		1939		1940		
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
																	Per cent
Sebago	50.0	50.0	70.0	50.0	30.0	30.0	10.0	10.0	30.0	10.0	30.0	10.0	50.0	30.0	50.0	30.0	33.8
Hindenburg	0.5	10.0	10.0	10.0	0.5	0.5	10.0	10.0	10.0	0.5	0.5	0.5	10.0	10.0	0.5	0.5	5.3
Richter's Jubel	0.5	10.0	0.5	0.5	10.0	10.0	10.0	10.0	0.5	0.5	0.5	0.5	10.0	0.5	0.5	10.0	4.7
Arnica	0.5	0.5	0.5	0.5	50.0	10.0	10.0	0.5	0.5	0.5	0.5	0.5	30.0	50.0	0.5	50.0	12.8
Houma	70.0	70.0	50.0	70.0	10.0	10.0	50.0	50.0	50.0	50.0	10.0	30.0	70.0	50.0	70.0	90.0	50.0
AA0–9	30.0	50.0	50.0	50.0	50.0	50.0	30.0	30.0	10.0	50.0	30.0	30.0	30.0	30.0	50.0	30.0	37.5
245–36	30.0	50.0	10.0	30.0	10.0	0.5	30.0	0.5	10.0	10.0	0.5	0.5	50.0	30.0	30.0	10.0	18.9
256–11	30.0	10.0	10.0	10.0	10.0	10.0	30.0	0.5	0.5	0.5	0.5	0.5	50.0	50.0	10.0	10.0	14.5
528–14	30.0	30.0	50.0	50.0	0.5	10.0	0.5	10.0	10.0	30.0	10.0	0.5	30.0	50.0	10.0	10.0	20.7
528–119	10.0	30.0	10.0	10.0	10.0	0.5	10.0	10.0	10.0	0.5	0.5	0.5	70.0	30.0	50.0	70.0	20.1
528–242	30.0	10.0	0.5	0.5	0.5	0.5	30.0	10.0	0.5	0.5	10.0	10.0	50.0	30.0	30.0	10.0	13.9
574–20	30.0	10.0	50.0	10.0	10.0	0.5	10.0	0.5	30.0	30.0	30.0	0.5	50.0	30.0	10.0	50.0	22.0
627–7	30.0	30.0	10.0	10.0	0.5	0.5	10.0	10.0	0.5	0.5	0.5	0.5	10.0	10.0	10.0	10.0	8.9
627–8	10.0	30.0	10.0	10.0	30.0	10.0	30.0	0.5	0.5	0.5	10.0	0.5	10.0	30.0	10.0	10.0	12.6
627–18	10.0	10.0	10.0	10.0	0.5	0.5	30.0	0.5	30.0	0.5	30.0	0.5	10.0	10.0	10.0	30.0	12.0
627–103	0.5	10.0	10.0	10.0	50.0	10.0	0.5	0.5	10.0	0.5	0.5	10.0	0.5	0.5	10.0	10.0	8.3
627–126	30.0	10.0	10.0	10.0	10.0	10.0	0.5	0.5	0.5	10.0	0.5	30.0	10.0	10.0	10.0	0.5	9.5
627–164	0.5	0.5	10.0	0.5	0.5	0.5	10.0	10.0	0.5	0.5	0.5	0.5	0.5	10.0	0.5	10.0	3.5
627–210	10.0	30.0	10.0	30.0	0.5	30.0	0.5	10.0	10.0	10.0	10.0	0.5	30.0	30.0	10.0	10.0	14.5
627–213	30.0	50.0	30.0	10.0	0.5	0.5	0.5	10.0	10.0	10.0	10.0	10.0	30.0	50.0	30.0	50.0	20.7
627–273	50.0	30.0	30.0	30.0	0.5	50.0	0.5	0.5	0.5	10.0	10.0	0.5	50.0	50.0	50.0	30.0	24.5
1037–5	10.0	0.5	10.0	10.0	0.5	0.5	30.0	10.0	70.0	50.0	10.0	10.0	50.0	50.0	50.0	50.0	25.7
Difference for significance at 5-per cent level																	7.6

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[a] Percentages of surface covered were taken in 6 classes. The data in the table are given in mean percentage.

Class	Extremes	Mean
	Per cent	*Per cent*
Trace	0–1	0.5
1	1–20	10.0
2	21–40	30.0
3	41–60	50.0
4	61–80	70.0
5	81–100	90.0

If the resistance of the varieties in these tests were judged by the data in table 2 the conclusions would be somewhat similar, but would not agree in every detail with those drawn from the data on type of pustule. Both Houma and Sebago showed a much greater variation in percentage tuber surface covered than they did in type of pustule. This was true also for the unnamed seedling varieties that might be considered intermediate in resistance, such as 574–20. The resistant varieties were more consistent.

TABLE 3.—*Variance for type of pustule, percentage of tuber surface covered, and the correlations between the two criteria*

Sources	d/f	Type of pustule	Percentage of tuber surface covered	Type of pustule and tuber surface covered
		Variances	*Variances*	*Correlation coefficients*
Varieties	21	3.40†	2,073.57†	−.85†
Replications	1	0.34	159.57	−.99†
Places	3	8.80†	5,721.35†	−.90†
Years	1	8.59†	1,092.04†	−.99†
Varieties × places	63	0.37†	425.51†	−.33†
Varieties × years	21	0.22*	96.25	−.03
Places × years	3	4.07†	156.10	−.47†
Varieties × places × years	63	0.46†	235.99†	−.38†
Error	175	0.11	118.37	......

* = Significant.
† = Highly significant.

Again taking Hindenburg as the standard for resistance and twice the standard error of a difference between means as indicating significance, Hindenburg, Richter's Jubel, Arnica, and selections 7, 8, 18, 103, 126, and 164 from the cross 627, are in the same class. It should be noted that only 4 of these—Hindenburg, Richter's Jubel, 627–7, and 627–164—were found in the most resistant class when judged by the type of pustule produced.

VARIANCES AND CORRELATIONS

The variances for type of pustule and percentage of tuber surface covered, and the correlations between these two criteria, are given in table 3. There was a highly significant difference between the means of the pustule types for varieties, places, and years, but the variance for replication was not significant. The interactions for variety × place and place × year were highly significant also, but the variance for variety × year did not greatly exceed the 5-per cent level. A comparison of the variances for percentage of tuber surface covered indicated highly significant differences for varieties, places, and years, but not for replications. Among the first order interactions only the variance for variety × place was significant.

Highly significant correlation coefficients were found between type of pustule and percentage of tuber surface covered for all but one of the sources of variability calculated.

DISCUSSION

It is evident that there are very significant differences between varieties of potatoes in their reactions to scab infection, and that comparative differences were fairly constant for the 22 varieties tested in 4 widely separated gardens. This might indicate that physiologic forms of *Actinomyces scabies* did not play a very important part in these tests. The significant interactions between variety and place would indicate, however, either physiologic forms or the influence of other environmental factors or both. The high interaction between place and year for type of pustule is evidence in favor of environmental factors rather than different physiologic forms, as it is highly improbable that the population of scab organisms would change its genetic nature from one year to the next.

Varieties selected in Maine showing the resistance of Hindenburg or Richter's Jubel have a good chance of being resistant in other parts of the country, but, because of the probability of the presence of physiologic forms and of the certainty of environmental influences, they should be tested in a wide range of conditions and over a period of years before generalized statements concerning their reactions are made.

SUMMARY

The 22 potato varieties tested in 4 potato-scab gardens show highly significant differences in their reactions to the common scab organisms for pustule-type and tuber-surface coverage.

The type of pustule and the percentage of tuber surface covered with pustules were highly correlated, indicating that in the majority of cases the relative reactions of the varieties to scab infection could be estimated by either criteria.

In general, the varietal reactions were quite constant for the 2 years and for 4 places. The highly resistant varieties showed less tendency to vary between years or between places than the slightly resistant or susceptible ones.

The interactions between variety and place are significant and could be due to environmental factors or to physiologic forms or to both. The highly significant interaction for pustule type between place and year indicates the influence of environment rather than different physiologic forms.

From the potato breeding point of view it seems possible to select varieties highly resistant to common scab in Maine and have them show similar resistance in other sections of the country, but before generalized statements of their reactions are made, they should be tested in a wide range of environmental conditions.

EFFECT OF ENVIRONMENT ON THE PREVALENCE OF SOIL-BORNE RHIZOCTONIA[1]

O. H. Elmer

(Accepted for publication March 11, 1942)

The rhizoctonia disease of potatoes, caused by *Corticium vagum* B. and C., attacks the potato plant through soil-borne mycelium or mycelium produced from soil-borne or tuber-borne sclerotia. Under eastern Kansas conditions this disease is characterized by local necrotic lesions on underground stems and stolons. A decrease in yield results from these lesions and from the delayed emergence and, consequently, the shortened growing season caused by the rotting off of infected sprouts prior to their emergence. The growing season for the spring potato crop in eastern Kansas ends in early July because the temperature is then so high as to stop growth and the plants die.

Tuber-borne Rhizoctonia sclerotia generally are recognized as being the more frequent source of infection of the potato. Prior to and in the early years of this investigation, evidence was obtained indicating that considerable damage to potatoes also may be caused by soil-borne Rhizoctonia.

The object of the investigations herein reported was to determine the relative importance of soil-borne Rhizoctonia as a cause of injury to the potato plant. Studies were made on the effect of soil moisture, including the drying effect of temperature and wind, on the ability of Rhizoctonia to persist from year to year in potato field soils.

METHODS

The prevalence of Rhizoctonia in potato-field soils was measured by the frequency with which soil-borne Rhizoctonia produced infections to experimental potato plants. The potato plants used in this investigation were produced from rhizoctonia-sclerotia-free tubers that had in addition been treated with an effective seed-potato treatment. Seed-potato treatments consisted of a 5-minute soak in hot formaldehyde solution, a 90-minute soak in 0.1-per cent solution of bichloride of mercury, or a 10-minute soak in a 0.2-per cent solution of bichloride of mercury acidulated with one per cent hydrochloric acid. Rhizoctonia infections that developed on potato plants produced from the treated sclerotia-free tubers were believed to have been initiated by the soil-borne organism. The prevalence of such infections is assumed to serve as an index on the prevalence of Rhizoctonia in the field soils.

Experimental plantings of sclerotia-free, treated potatoes in commercial fields were made anually during the seasons 1928 to 1940, inclusive. The seed pieces were planted in duplicated 50-foot rows in March during planting of the commercial crop. The soil-rhizoctonia test plots were established in 3 commercial fields in 1928, and thereafter in an average of 15 such fields.

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[1] Contribution No. 414, from the Department of Botany, Kansas Agricultural Experiment Station.

During the first 4 years of this investigation rhizoctonia-infection data were obtained when the potatoes were harvested in July, but difficulty was experienced in ability definitely to recognize rhizoctonia lesions at this time, because the plants were reaching maturity and some of them were dying. In 1932 and thereafter, rhizoctonia-infection data were obtained in May when the plants were beginning to bloom. Maximum rhizoctonia infection, including infections on underground stems and stolons, has occurred by this time, and the lesions can be recognized easily. In obtaining these data the plants, including the newly developing tubers, were removed from the soil and the infection data were recorded.

During the seasons 1931 to 1940 rhizoctonia-sclerotia-infected nontreated tubers were planted in rows adjoining the rhizoctonia-free potatoes. The infections that developed on these control plants served as an index on the ability of Rhizoctonia to produce lesions on potato plants under the prevailing environmental conditions.

Weather records from 1927 to 1939 showing the factors affecting soil-moisture content of potato fields in eastern Kansas are presented in this report. Special attention has been given to annual precipitation and to rainfall, temperature, and wind during July and August. These weather conditions are of primary importance in determining the soil-moisture content of the soils of commercial eastern Kansas potato fields during July and August, when soil-moisture content becomes the lowest.

RESULTS

The results obtained during the 13 years of this investigation show that environmental conditions are highly important in determining how successfully soil-borne Rhizoctonia can persist from year to year in eastern Kansas field soils.

The commercial potato crop in eastern Kansas matures and usually is harvested in early July. Throughout the rest of the summer the fields usually remain fallow. Summer temperature in this area is too high for the production of rhizoctonia sclerotia as is disclosed by the fact that they are rarely found here on tubers of the early summer crop. Thereafter the fungus must consequently exist saprophytically as soil-borne mycelium. In this state its persistence depends on the presence of sufficient soil moisture to prevent death from desiccation. The springtime prevalence of soil-borne Rhizoctonia in these fallow fields indicates to what extent this fungus is able to persist in such field soils during the preceding summer.

A second crop of potatoes or a crop of rhizoctonia-susceptible vetch, cowpeas, or soybeans, sometimes planted about August 1, promotes the ability of this organism to persist in potato fields during hot, dry summers.

Effect of Soil Moisture Content During the Summer on Survival of Rhizoctonia in the Soil

The soil-moisture content of fallow potato fields during the summer months is largely determined by the total annual precipitation and the July-August rainfall, by summer temperatures, and by air movement, which may be measured as total miles of wind per month. Records of these environmental factors for 1927 to 1939 inclusive, are presented in table 1. The summer temperatures recorded are the average daily maxima for the month.

TABLE 1.—*Effect of environment on prevalence of soil-borne Rhizoctonia*

Year	Average maximum daily temperature (degrees F.)		Precipitation (inches)			Wind miles			Per cent[a] infection
	July	August	July	August	Annual	July	August	Total	
1927	86.7	80.0	6.23	6.80	43.80	5,309	4,737	10,046	....
1928	87.8	87.6	2.75	4.91	32.20	5,330	5,454	10,784	53.2
1929	88.6	89.5	1.70	2.64	32.81	5,695	5,297	10,992	50.4
1930	93.9	90.9	1.57	2.88	33.89	6,035	5,089	11,124	21.3
1931	91.8	85.0	3.41	6.48	36.79	5,394	4,757	10,151	36.2
1932	90.6	89.4	2.89	7.82	28.36	6,314	6,314	12,628	32.1
1933	92.9	86.3	2.47	3.50	22.24	6,364	5,869	12,233	12.5
1934	102.0	97.0	2.87	1.71	27.30	6,907	6,548	13,455	3.0
1935	99.7	91.6	0.03	3.98	37.31	6,205	6,160	12,365	1.1
1936	102.5	101.2	1.11	0.57	22.63	6,599	6,544	13,143	4.9
1937	95.5	96.4	1.65	3.51	19.43	6,287	6,384	12,671	3.1
1938	94.0	96.2	3.97	2.15	29.01	5,610	7,299	12,909	1.1
1939	99.1	89.3	1.26	3.51	22.33	6,759	6,198	12,957	1.3
1940	....	....	....	....	....	....	....	....	3.8

[a] From Rhizoctonia that persisted in the soil through the preceding summer.

Meteorological data from the Topeka station of the United States Weather Bureau, presented in table 1, show that the annual precipitation and the July–August rainfall were relatively high and that temperature and total wind mileage were relatively low during July and August of 1927 to 1932, inclusive.

The annual rainfall at Topeka for the period 1927 to 1932, inclusive was respectively, 43.80, 32.20, 32.81, 33.89, 36.78, and 28.36 inches. The July–August rainfall during these years was, respectively, 13.03, 7.66, 4.34, 4.45, 9.89, and 10.71 inches. The maximum daily temperature for July and August averaged below 90° F., and total miles of wind for the same months varied from 10,046 to 11,124 miles.

As a result of these environmental conditions, eastern Kansas potato-field soils did not become excessively dry and a considerable amount of soil-borne Rhizoctonia was able to persist during the summer months. It is shown in table 1 that 53.2, 50.4, 21.3, 36.2, 32.1, and 12.5 per cent, respectively, of the experimental potato plants became infected with Rhizoctonia during the springs of 1928 to 1933.

Weather conditions in eastern Kansas during 1933 to 1939, inclusive, differed widely from those of the first 6 years of this investigation. Annual precipitation during 1933 to 1939, inclusive, with the exception of 1935 was relatively low; only small amounts of rain fell during the summer months of these years. The annual rainfall during these 7 years was, respectively, 22.24, 27.30, 37.31, 22.63, 19.43, 29.01, and 22.33 inches. Rainfall during July and August of each of these years was, respectively, 5.97, 4.58, 4.01, 1.68, 5.00, 6.12, and 4.77 inches. Summer temperatures were relatively high during these years, and there was considerably more wind than during the first 6 years of this investigation. The average daily maximum temperature for July and August in 1933 to 1939, respectively, was 89.6, 99.5, 95.6, 101.9, 95.9, 95.1, and 94.2° F., and total miles of wind during July and August of these years varied from 12,233 to 13,455 miles.

As a result of the deficient rainfall, the high summer temperatures, and the relatively windy weather during 1933–1939, field soils became excessively dry. Sub-soil moistures became depleted during the consecutive years of drought and the meager rains that fell soon evaporated due to the then prevalent dry weather conditions.

Infection data obtained during the springs of 1934 to 1940, inclusive, (table 1) indicate that very little soil-borne Rhizoctonia was able to survive in eastern Kansas potato fields during the summers of 1933 to 1939. The average number of potato plants infected by soil-borne Rhizoctonia during the succeeding springs of 1934 to 1940 was, respectively, 3.0, 1.1, 4.9, 3.1, 1.1, 1.3, and 3.8 per cent.

Sclerotia-infected, nontreated potatoes, planted as controls in rows adjoining the sclerotia-free treated tubers, produced many rhizoctonia-infected plants. The percentages of plants that became infected with the seed-borne fungus in the tests of 1930 to 1939 were, respectively, 85.5, 95.6, 92.2, 88.6, 94.4, 90.7, 96.4, 99.3, and 92.3. These data provide evidence that the low infection percentages by soil-borne Rhizoctonia during the later years of this investigation were not due to unfavorable environmental conditions but to the presence of only small amounts of Rhizoctonia in the field soils.

Effect of Living Host Plants on Survival of Rhizoctonia in the Soil

Data obtained during this investigation indicate that the survival of soil-borne Rhizoctonia in relatively dry soils is greatly facilitated when living host plants of this pathogen are present.

It was repeatedly noted that the Rhizoctonia content of potato field soils was higher when a fall crop of potatoes or when such rhizoctonia-susceptible fertility crops as vetch, or cowpeas, had been grown in those fields the preceding summer and fall. Sclerotia-free, treated tubers were planted in the spring of 1936 in 3 fields in which a fall crop of potatoes had been produced in 1935. Soil-borne Rhizoctonia developed the following spring on, respectively, 15.0, 17.5, and 58.3 per cent of the experimental potato plants in these 3 fields. In contrast, only 4.9 per cent infection occurred in those commercial fields that, in 1935, had not produced a fall crop of potatoes. The field in which 58.3 per cent infection occurred had been planted in August, 1935, with untreated tubers severely infected with rhizoctonia sclerotia.

DISCUSSION

Prevalence of soil-borne Rhizoctonia in the potato fields of the Kansas

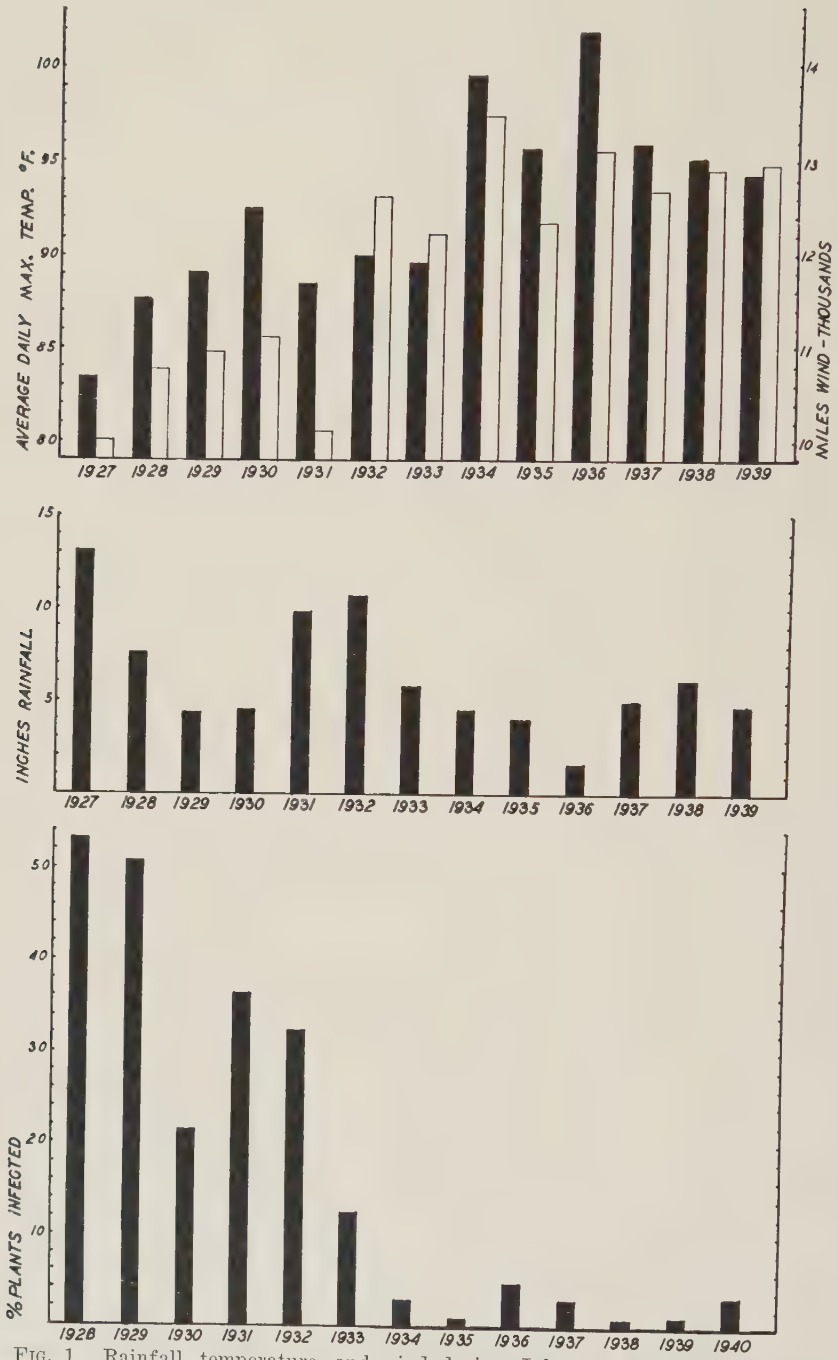

FIG. 1. Rainfall, temperature, and wind during July and August and subsequent prevalence of soil-borne Rhizoctonia. The graph indicates the relatively high rainfall and the relatively low temperatures (solid bar) and wind (light bar) during July–August, 1927–1932, when much Rhizoctonia was able to persist in potato field soils. Relatively low rainfall, high temperature, and windy weather during July–August, 1933–1939, were followed by a greatly reduced rhizoctonia content of the field soils.

River Valley varied greatly during the 13 years of this investigation. The data indicate that, under eastern Kansas conditions, Rhizoctonia can persist saprophytically in fallow soil only during summers when the soil does not become excessively dry.

The correlation between summer weather conditions and the survival ability of Rhizoctonia in potato-field soils is illustrated in figure 1. This graph illustrates how the abundant summer rainfall of the early years of this investigation were accompanied by low summer temperature and relatively little wind, and, inversely, how low rainfall in July and August of the later years was accompanied by high summer temperature and high winds. The graph further illustrates how soil-borne Rhizoctonia was comparatively abundant following moist and relatively cool summers and how its prevalence decreased following summers when the field soil became excessively dry.

The periodic dying out of soil-borne Rhizoctonia, induced by environmental conditions, is important to potato growers in eastern Kansas. Infections from soil-borne Rhizoctonia may be prevalent in potato plants following such cool, moist summers as those of 1927 and 1928, but injury from this source is of little importance following summers of low soil-moisture content. The prevalence of soil-borne Rhizoctonia in eastern Kansas can be approximately predicted from the relative soil moisture content of the preceding summer.

The potato growers' most serious losses from Rhizoctonia usually result from seed-borne inoculum. Observations during the course of this investigation indicated that severity of injury to affected plants is much the same, whether from tuber-borne or soil-borne Rhizoctonia, but that the infections from the seed-borne organisms are usually more prevalent. In regions like eastern Kansas, where soil-borne Rhizoctonia may periodically be practically eradicated because of environment, effective seed-potato treatments are valuable not only in preventing infection of the current season's crop but also in preventing reinfestation of the soil with this fungus.

SUMMARY

Wide differences were noted in the prevalence of soil-borne Rhizoctonia in Kansas potato fields during the 13 years of this investigation. Rhizoctonia was able to survive in the soil only in those years when there was sufficient summer rainfall to prevent desiccation and death of the organism in its mycelial stage. Sclerotia were absent during the critical summer months, because their formation requires cooler weather than usually occurs in Kansas in July and August.

The rhizoctonia organism can persist parasitically on infected host plants during summers when the soil is so dry that it cannot survive saprophytically.

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CONTROL OF FUNGI IN MUSHROOM CASING SOIL BY STERILIZATION WITH CHLOROPICRIN[1]

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INTRODUCTION

Mushroom beds are covered with soil 1 inch deep in order to induce the formation of mushrooms. An increasing number of growers are employing soil sterilization as a regular part of their control practice. Sterilization of this soil (casing soil) is aimed principally at eradication of *Mycogone perniciosa* Magnus, the organism causing the destructive "bubble" disease. This organism has become so widespread in areas where mushrooms have been grown intensively that it is almost impossible to obtain soil that is entirely free of it. Soil sterilization has been particularly necessary for growers who have mushrooms on the beds in early fall and late spring when the "bubble" disease becomes most serious.

Increased attention to the control of mushroom diseases has indicated that the casing soil may also be the avenue of entrance for other organisms besides *Mycogone perniciosa*. This is particularly true of soils contaminated with trash or drainage from mushroom houses. Among the organisms that may be introduced with contaminated soil are the following: *Verticillium malthousei* Ware, causing spotting and malformation of the mushrooms: *Dactylium dendroides* (Bull.) Fr., causing soft decay; *Pseudobalsamia microspora* Diehl and Lambert, the "truffle," which may be introduced into the compost when infested soil is added to it or which may be carried in with the casing soil (1). The less important green molds *Trichoderma* sp. and *Penicillium* sp., which may become serious if the beds are not properly managed, are also common inhabitants of the soil.

Under certain conditions the opportunity exists for pests like mites and springtails to find their way into the houses via the casing soil. Infestations of the long-legged mite, *Linopodes antennaepes* Banks have been traced to the casing soil. The frequency with which such pests get into the houses by this means is not definitely known, yet the possibility must be considered in a strict sanitation program.

In the widest sense, then, soil sterilization should be regarded as having the general aim of eradicating all the injurious fungi and pests. It must be admitted, however, that, ordinarily, the miticidal and insecticidal properties of the sterilization treatment are unimportant.

Steam sterilization has proved satisfactory as a fungicidal as well as an insecticidal measure. Its chief disadvantages to most growers are cost of equipment and necessity of hiring it. Very infrequently certain soils are damaged by the steam treatment. This toxic effect also has been observed in greenhouse soils. Lambert has pointed out the danger of overheating casing soil (5).

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[1] The writer is indebted to J. W. Sinden and C. A. Thomas of the Pennsylvania State College for suggestions in the course of the study and for criticism of the manuscript.

Beach has recommended sterilization with formaldehyde (1). The necessary technique is simple and inexpensive, and is very effective against *Mycogone perniciosa.* Consequently, it has become widely popular among mushroom growers. Formaldehyde is not an insecticide, though, admittedly, this is not of prime importance. Some soils, particularly those in which nitrogen-fixing plants have been grown, respond unfavorably to formaldehyde treatment. In these cases, the soil becomes predisposed to the development of an excessive amount of green molds or the mushrooms fail to form properly. This type of damage can, however, be avoided by using the proper kind of soil. Formalin is now somewhat difficult to secure because of war priorities.

The success of chloropicrin as a nematocide, insecticide, and fungicide has suggested its use for mushroom casing soil (2, 3, 7). It volatilizes rapidly into a powerfully lethal ''tear gas.'' Accordingly, research was undertaken to determine its possible efficacy in sterilizing casing soil.

EXPERIMENTAL CONTROL OF FUNGI IN CASING SOIL

Since eradication of the spores of *Mycogone perniciosa* is the primary purpose of sterilization, several studies were made on the susceptibility of this organism to chloropicrin. Four cubic feet of soil were inoculated with *Mycogone* spores by mixing in macerated tissue of a large number of mushrooms badly affected with ''bubble'' disease. This soil was divided into 4 boxes, each holding 1 cu. ft. Three of the boxes were treated with chloropicrin at the rate of 2, 3 and 5 ml., respectively. A single injection, 6 in. deep, was made in the center of each box. One box was left untreated. The surface of all the boxes was watered, then covered with several layers of cardboard. After 3 days the cover was removed. The gas was still very strong at this time in the treated boxes. In 10 days all trace of it had disappeared. The contents of each box was used to case 2 duplicate trays, each containing about 7 sq. ft. of mushroom bed. The trays were then put under ordinary cold frames in preparation for bearing. The experiment was conducted in the spring, and the afternoon temperature in the frames was frequently as high as 70° F.

On the first break all the mushrooms on the untreated plots were severely malformed, showing the characteristic puffball-like symptom induced by *Mycogone perniciosa.* On the remaining 6 plots no diseased mushrooms appeared. The infected plots were immediately discarded. Subsequently, mushrooms were picked off the remaining plots for the next month. No diseased mushrooms appeared on any of the plots.

It is evident that under the conditions of the experiment 2 ml. of chloropicrin per cu. ft. sufficed to kill the spores of *Mycogone perniciosa.* All the treated plots seemed to react in the same way. A good number of high-quality mushrooms were picked from each tray.

The effect of chloropicrin on other fungi common to mushroom beds was similarly determined. Soil was inoculated with spores of *Verticillium malthousei, Dactylium dendroides,* and *Pseudobalsamia microspora.* Two trays were used for each species, one containing soil treated at the rate of 2 ml. of chloropicrin per cu. ft. and the other untreated.

The untreated plot containing *Verticillium* spores produced mushrooms that were about 80 per cent spotted on the first break. No spotted mushrooms appeared in the treated soil. It is interesting to note that Godfrey has reported control of *Verticillium alboatrum* on strawberries and Verticillium wilt of tomatoes with chloropicrin (3). The untreated tray in which infection occurred was covered with glass to increase the humidity. On the next break all the mushrooms were spotted or malformed. The control was disease-free.

No sign of the *Dactylium* appeared in the untreated plot until the second break, when it appeared in the corner of the plot. The tray was then heavily watered and covered with glass. In 2 weeks' time the entire tray was covered with a luxuriant growth of Dactylium mycelium, and mushrooms that emerged were attacked. The fungus did not appear in the treated tray.

In the trays cased with soil inoculated with *Pseudobalsamia,* truffle did not appear either in the treated or non-treated plot. Hence, no conclusion can be drawn.

FUMIGATION OF CASING SOIL IN BINS

For large-scale sterilization it was decided to treat the soil in roughly constructed bins erected on the composting ground in the rear of the mushroom houses (Fig. 1). Such a procedure should secure a uniform distribution of the chloropicrin and guarantee the best possible confinement of the gas. A bin approximately 9 ft. wide, 11½ ft. long and 3 ft. high, holding

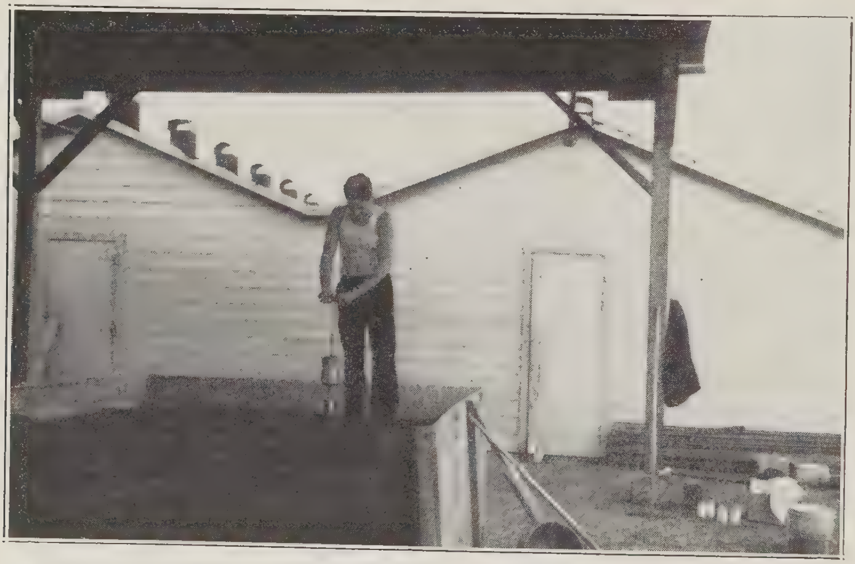

FIG. 1. Injection of chloropicrin into casing soil in wooden bin.

enough soil to case a standard house of about 4000 sq. ft. of bed space, was made with the sides detachable so that they could be broken down and either transported or stored. Wooden or iron supports were propped against the sides when the bin was full. The bin was erected immediately after the houses were filled.

An interval of 3 to 4 weeks ordinarily intervenes between filling and casing, ample time to allow escape of the gas. As usual, the soil was screened through a 1-in.-mesh screen before placing in the bin. A bottom layer of soil 1 ft. deep was thrown into the bin, leveled off, and marked out into 1 ft. squares. Five ml. of chloropicrin were injected 4 inches deep into the center of each square. A second and third layer of soil, each 1 ft. deep, were treated in the same manner. About 5½ lb. of chloropicrin were used for the bin. When the latter was full the surface was watered to a depth of about an inch and covered with the glue-coated paper recommended by Godfrey (3). After 3 days the cover was removed. A week later the pile was turned over. Subsequent experiments proved this operation unnecessary, since the gas normally escaped within 2 weeks.

The soil used in this experiment came from a field adjoining a mushroom plant. The grower in whose houses the experiments were done had used soil from the same source for years and had finally been compelled to sterilize it because of serious losses from the "bubble" disease.[2] A binful of this naturally infested soil was treated in the above manner. A second pile was left untreated. A third was treated with formaldehyde, as recommended by Beach, and a fourth was steam-treated. Four houses were subsequently cased with the 4 piles treated in different ways. The houses were kept in bearing 3 months, during which time careful observations were made. Many bubbles appeared on the first break in the house containing non-treated soil. On subsequent breaks the loss resulting from Mycogone infection became quite serious. An unusually long hot spell increased the severity of infection, so that the difference between this house and the houses containing treated soils was very conspicuous. In these practically no bubbles appeared in the first 3 weeks. A few appeared in later breaks, evidently because of reinfection. Apparently all 3 of the treatments, chloropicrin, steam, and formaldehyde, were equally effective. While production records were not kept in these houses, it was impossible to observe differences in the variously treated houses.

A number of houses were subsequently cased with soil treated with chloropicrin in the same manner. In all cases a good control of the bubble disease was effected. It proved unnecessary to build a bin for each pile; instead, a binful was treated, then broken down after 48 hours, and moved over and assembled for the next lot. The end of the bin into which the soil is thrown may be built up with 1-ft. wooden boards as the soil is put in or it may be left entirely open. As Godfrey has pointed out, the water seal is unnecessary when the gas-impervious paper is used. As a matter of fact,

[2] The experiments were carried out in the houses of J. B. Swayne, Kennett Square, Pa.

soil treated in bins may simply be covered with burlap, newspapers, or building paper and then wetted down.

Production records were kept in two houses, one of which contained soil chloropicrin-treated, the other, soil treated with formaldehyde. The number of 10-lb. baskets taken out of each house during the 3 months of production was recorded. The soil treated with chloropicrin yielded 1.7 lb. per sq. ft. The formaldehyde-treated soil yielded 1.4 lb. per sq. ft. The difference cannot be attributed to the treatments. No replications were made. The results, however, show that chloropicrin does not impair the productive capacity of the soil. Previous observations tended to establish this point.

Increased fertility of the soil, sometimes noticed as a result of partial sterilization with chloropicrin, has not been obvious in these studies. One reason for this may be that the soil is not a source of nutrients for the mushroom. In mushroom culture the sole object of soil sterilization is the elimination of harmful organisms. If this be achieved without damaging the soil, the treatment may be regarded as satisfactory.

An attempt was made to treat soil in loose piles, but was promptly abandoned because it was difficult to obtain uniform distribution of the chloropicrin in loosely shifting soil. One Kennett Square grower has succeeded in controlling the disease by treating the soil in loose piles. This practice is, however, rather uncertain and is not at all recommendable.

In the preliminary experiments it was shown that spores of *Mycogone* were killed by a dosage of 2 ml. per cu. ft. Five ml. per cu. ft. were used in the bins in order to insure a lethal concentration under the variable conditions that might exist in the field. Experience with the bins indicated that confinement of the gas was good enough to permit a reduction in dosage. The soil for several houses was treated at the rate of 3 ml. per cu. ft. Observations on these houses indicated clearly that 3 ml. per cu. ft. were adequate.

FIELD TESTS

Fumigation of soil in bins is somewhat troublesome because the soil must be treated in 1 ft. layers. This labor is not prohibitive, since the soil must be piled on the composting ground, anyway. It would be simpler still, however, to inject the soil directly in the field prior to screening. Ordinarily, the soil is disked and then screened. It is thus in loose condition and suitable for treatment. Partial sterilization of the soil directly in the field has been satisfactorily accomplished for other purposes (2). A preliminary experiment was conducted to test this point. Two plots, 6 feet square, were disked to a depth of 8 inches and inoculated with spores of *Mycogone*. A ditch 1 foot wide and a foot deep was dug around each plot. Many lumps and clods of soil were lying freely on the surface, but no attempt was made to crush them, since the grower would not find it practical to do this. After infestation, the soil in each plot was mixed by turning with a shovel. From one of the plots about ½ cu. ft. of soil was removed for subsequent use as a check. The plots were then marked out into 1-foot squares, and the center of each square injected with 3 ml. of chloropicrin to a depth of 3 inches. Because of cost a heavier injection was not applied. Most growers use no more than the top 6 or 8 inches of soil. Both plots were sprinkled with water to create a water seal. One plot was covered with canvas and the edges buried all around in soil. The other was left uncovered. By the next day the surface of the uncovered plot had dried out. After 3 days the canvas was removed. The gas was distinctly detectable at that time. None could be detected in the uncovered plot. The daily afternoon temperature during this experiment was about 80° F. The soil of both plots was again thoroughly mixed. Enough was removed from each plot to case two trays similar to those employed in the first experiments. An added tray was cased with soil removed from one of the infested plots prior to treatment.

The trays were put into an empty house. The mushrooms on the non-treated soil began to emerge as typical bubbles and the tray was discarded. The soil from the treated, uncovered plot yielded mushrooms in both trays, which were about 35 per cent infected. These, too, were discarded. The soil from the plot covered with canvas after the treatment yielded disease-free mushrooms for 2 breaks, when they, likewise, were discarded.

It is obvious that adequate confinement of the gas is impossible with a water seal. This is true particularly during hot weather, when the water tends to evaporate readily and the gas volatilizes more quickly. On the other hand, the water seal and canvas covering were effective. Undoubtedly, the former can be eliminated. Godfrey has reported the water seal entirely unnecessary when the gas-impervious glue-coated paper is used. He estimates that this paper would cost not more than 2¢ per sq. ft. and it may be used repeatedly (3). The only objection to the use of strips of glue-coated paper is that they are difficult to keep in place on a windy day. This actually was the experience when they were tried in an experiment. On the other hand, canvas is heavier and may be had in large pieces. Whatever type of cover is used, a tight seal should be made around the edges. Three ml. per square foot suffices if the confinement of the gas is good. The importance of good confinement cannot be over-emphasized. No attempt was made to determine the depth of penetration of the gas, though it seems clear that it penetrated the top 8 inches.

Field treatment is a comparatively simple matter. Enough soil can be treated and covered in an hour to case a standard house. This amount of soil is contained in a plot about 25 ft. square if the top 6 inches are removed. It requires about 7 lb. of chloropicrin to treat this area in the field at the rate of 3 ml. per sq. ft. Half this amount is required for an equal amount of soil in the bins. It must be emphasized that the efficacy of the field treatment will depend on the degree of confinement of the gas. The surface of the field should be leveled off with a rake before the injections are begun. Not all the soil need be treated at one time. A plot may be treated one day and covered. After 48 hours, the cover may be transferred to the next plot. In this way a relatively small amount of cover can be successively used for a large area.

The soil should not be allowed to remain in the field very long after the cover is removed because of danger of recontamination. It is inadvisable to treat the soil too far in advance of its use. Ordinarily, injections may be begun when the houses are filled. A week later the soil may be removed and piled on the composting ground, as usual.

FUMIGATION OF EMPTY HOUSES

Several trials were made to determine the value of chloropicrin as a fumigant. Formaldehyde or sulphur-dioxide ordinarily are used for fumigation of mushroom houses between crops. It was observed that chloropicrin did not evaporate rapidly enough when poured into a shallow pan. Rapid evaporation did occur when the chloropicrin was slowly poured along the length of the elevated walkway in the middle aisle. A gas mask was used during this operation. The fungicide was applied at the respective rates of $\frac{1}{8}$, $\frac{1}{4}$, and $\frac{1}{2}$ lb. per cu. ft. Spores of *Mycogone, Dactylium,* and *Trichoderma* were not killed by any of the fumigations. It was not considered advisable to use more than a half pound per thousand cubic feet because of the prohibitiveness of the cost.

CONCLUSION

Sterilization of casing soil with chloropicrin is a practical procedure that can be easily adapted to cultural practice. The fungicidal powers of chloropicrin cannot be doubted.[3] As a matter of fact, the field treatment with chloropicrin is as simple as sprinkling with Formalin and requires little effort or time. The grower may experience a little trouble with the special injectors at first until he learns the technique. It is essential to secure an adequate confinement of the gas. This may be easily managed by the use of a canvas. Merely wetting the surface of the soil after the treatment will not prevent rapid escape of the gas, especially in warm weather. Bin treatment is somewhat more troublesome than field treatment, but it is not at all prohibitively so. It is true, however, that only half the amount of chloropicrin used in the field is necessary when the soil is treated in bins. Soil should not be treated when the outside temperature drops below 60° F. Wet soils must be avoided because of the very limited solubility of chloropicrin. A loose, moist soil is ideal. A gas mask is not needed when the soil is treated outdoors. The treatment can be carried out with almost no inconvenience to the operator. Soil for the winter refill must be treated during warm weather and stored in a clean place. It is entirely practicable to inject the chloropicrin into the soil as it is being piled up in the storage shed; the technique is very simple and requires but little extra effort. Since the stored soil will not be needed for 3 to 5 months, there will be a sufficient interval to permit the escape of the gas. A gas mask should be used if the storage shed does not admit a good supply of fresh air.

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[3] C. A. Thomas of the Pennsylvania State College has investigated the susceptibility of several species of mushroom house pests to chloropicrin. His studies were made coincidently with those reported in this paper and were carried out under the same conditions. The results of this work will be reported in the Journal of Economic Entomology.

Actually, chloropicrin need replace formaldehyde only when it is suspected that mites or springtails are getting into the houses with the casing soil. One grower in North Carolina suffered material losses as a result of the damage caused by the long-legged mite, *Linopodes antennaepes.* He was able to eliminate the mite by fumigating his casing soil with chloropicrin. In instances of this kind, chloropicrin is, of course, more desirable than formaldehyde because of its insecticidal powers. At the present time Formalin is becoming somewhat difficult to obtain because of war priorities. Under these circumstances growers should not hesitate to use chloropicrin.

SUMMARY

Spores of *Mycogone*, *Verticillium*, and *Dactylium* were killed by chloropicrin used at the rate of 2 ml. per cu. ft. of soil. A method of treating soil in roughly constructed wooden bins is described. Three ml. of chloropicrin per cu. ft. gave good results in the bins. A method of treating soil directly in the field is described. In this method 3 ml. of chloropicrin are used for each square foot of surface. Good results are obtainable in the field if the gas be confined by an impervious cover. Soil treated with chloropicrin gave results comparable with those from soil treated with formaldehyde and steam. It is not economical to use chloropicrin for the fumigation of empty houses.

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A STRAIN OF TOBACCO-MOSAIC VIRUS CAUSING A NECROSIS AND SHRIVELING OF TOMATO FOLIAGE

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INTRODUCTION

At times during the past 6 years, greenhouse tomatoes at Beltsville, Maryland, have been affected by a mosaic disease causing necrosis and shriveling of the foliage. The disease has been observed only once in the field, but is known to have occurred as early as 1930 in greenhouses in Washington, D. C., and at Arlington, Virginia. On tomatoes it causes considerably greater injury than either the common green form of tomato (tobacco) mosaic (*Marmor tabaci* var. *vulgare* Holmes) or the yellow strain of the same virus (*Marmor tabaci* var. *aucuba* Holmes). It has been of particular interest because of its sporadic appearance in the greenhouse and the lack of evident sources of primary infection.

SYMPTOMS

Tomato

On tomatoes, the first symptom consists of a mottling much like that of the ordinary green tobacco mosaic, although of a slightly yellower type in young plants. This, however, does not resemble the bright, yellow mottle of the yellow or aucuba strain of tobacco mosaic. Within 12 to 15 days after inoculation, the older leaves begin to develop small, diffuse yellow areas, which show a minute, reddish-brown, necrotic stippling that gives these spots a characteristic russet-orange color (Fig. 1, B). These rusty patches gradually develop into large, pale-brown, papery spots that generally extend to the margins of the leaflets (Fig. 1, A). When so affected the leaflets are curled downward at the margins, twisted, and somewhat malformed (Fig. 2, A). Occasionally, an entire leaflet yellows rapidly, and fine, necrotic markings occur along the small veins (Fig. 1, C). This general yellowing of the older leaflets has been noted only in plants from 12 to 18 inches tall at the time of inoculation. It has occurred only when unusually low temperatures (65° to 70° F.) prevailed for 3 to 4 days just preceding the time when symptoms were first appearing.

As the disease progresses, the older leaves wither but remain attached to the plant. The withering of the foliage continues slowly up the stem and is readily confused with damage from fumigation or other chemical treatments (Fig. 2, B). There is no stem or petiole necrosis, and the new leaves show a green mottle with occasional reddish-brown necrotic areas that enlarge slowly. The affected leaflets are somewhat dwarfed and much curled and twisted. After they mature, these leaves gradually wither, but a fair amount of foliage always remains green at the upper portion of the plant. Only a few fruits are produced, but these are of normal appearance.

Leaf necrosis develops most rapidly at low temperatures. When held at temperatures ranging from 60° to 75° F., young, rapidly growing plants of 7 to 8 leaves have occasionally developed a general necrosis within 14 days after inoculation. The virus is readily transmitted by the ordinary leaf-rubbing technique or by brushing, handling, or pruning diseased and healthy plants.

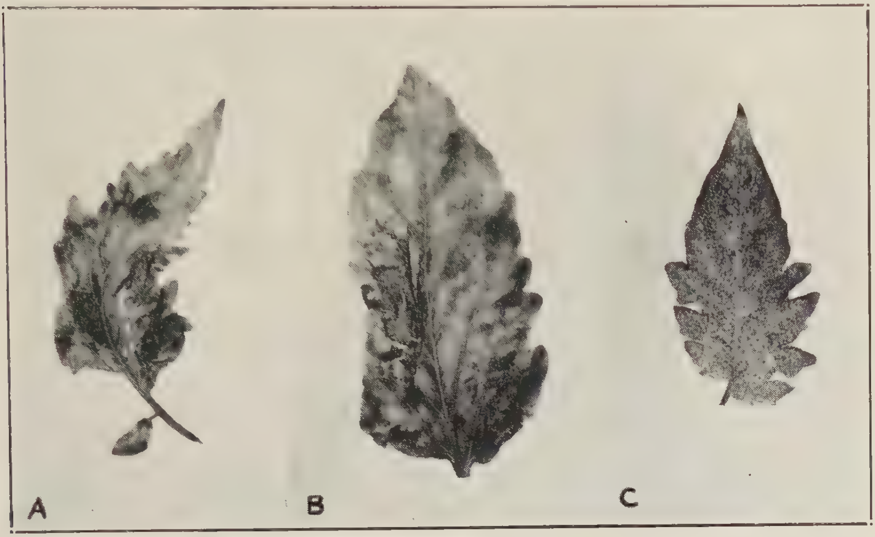

FIG. 1. Symptoms produced on tomato leaflets by the leaf-shriveling strain of the tobacco-mosaic virus. A, Typically curled and twisted leaflet showing yellowing and necrotic spots near the margin; B, leaflet showing necrotic spots and the russeting that precedes severe necrosis; C, leaflet entirely yellowed and covered with a fine, stippled necrosis.

Other Host Plants

As far as determined, the host range of the leaf-shriveling virus has been the same as that of tobacco mosaic. When tested in parallel series of inoculations on various wild species of *Lycopersicon,* the symptoms have been the same on all susceptible species. Those species showing a complete suppression of symptoms, when infected with the tobacco mosaic virus, have shown the same reaction to the tomato virus. The following species have been tested: *Lycopersicon pimpinellifolium* (Jusl.) Mill., *L. hirsutum* Humb. and Bonpl., *L. hirsutum* f. *glabratum* C. H. Mull., *L. peruvianum* (L.) Mill., *L. peruvianum* var. *dentatum* Dun. and *L. glandulosum* C. H. Mull.

On the Samsun variety of turkish tobacco (*Nicotiana tabacum* L.) the symptoms have always been the same as those of ordinary tobacco mosaic. At times, the tomato virus appears to produce a slightly yellower green of the entire leaf, but the pattern of the mottling is that of ordinary tobacco mosaic; no consistent differences in symptoms have been observed. There is no necrosis of leaf or stem and no yellow mottling of the aucuba type at any stage of growth.

On *Nicotiana rustica* L., *N. paniculata* L., and *N. quadrivalvis* Pursh., the symptoms also are like those of tobacco mosaic. The same is true of *N. glutinosa* L., where primary lesions are produced like those of tobacco mosaic, and the virus generally remains localized in the inoculated leaves. On *N. glauca* Graham, it differs from ordinary tobacco mosaic in the production of minute, superficial, reddish-brown, stippled lesions on the inoculated leaves but there is no systemic necrosis or mottling, although the virus becomes systemic.

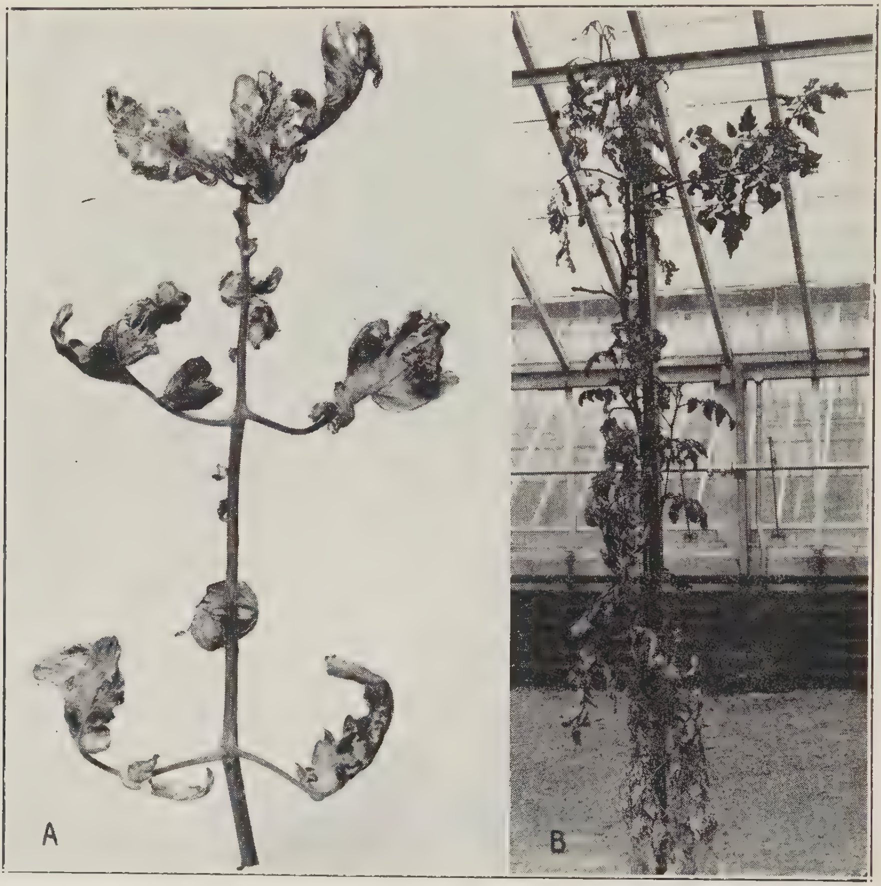

FIG. 2. A, Tomato leaf showing curling, twisting, and necrosis of the leaflets typical of the leaf-shriveling virus; B, tomato plant showing shriveling of the older foliage.

On *Nicotiana sylvestris* Speg. and Comes, the tomato virus behaves like the aucuba type of mosaic in producing dark-brown, circular, necrotic primary lesions. It has not become systemic on this host except in the case of occasional young plants grown at high summer temperatures (90°–95° F.) in the greenhouse. In these plants there was a veinal necrosis and eventual death of all but the youngest leaves.

On pepper (*Capsicum frutescens* L.), *Physalis angulata* L., *Physalis* *pubescens* L., *Physalis heterophylla* Nees., and *Physalis alkekengi* L. the symptoms are like those of tobacco mosaic and the same is true for *Datura stramonium* L., where the tomato virus produces primary local lesions but does not become systemic. On Scotia bean (*Phaseolus vulgaris* L.) the primary lesions also are like those of tobacco mosaic.

No infection has occurred in cucumber (*Cucumis sativus* L.), muskmelon (*C. melo* L.), watermelon (*Citrullus vulgaris* Schrad.), beet (*Beta vulgaris* L.), lettuce (*Lactuca sativa* L.), celery (*Apium graveolens* L.), or pokeweed (*Phytolacca decandra* L.).

RELATIONSHIP TO THE VIRUS OF TOBACCO MOSAIC

Physical Properties

As far as determined, the physical properties of the leaf-shriveling virus are the same as those of ordinary tobacco mosaic. A number of comparative determinations on young plants of tomato, turkish tobacco, and *Nicotiana glutinosa* have shown that the thermal destruction point of both viruses lies between 90° and 95° C. These trials were made with 2 cc. samples of the plant juices diluted 1 to 5 with tap water and heated for 10 minutes in a water bath controlled within ± 0.2° C.

Both viruses have produced a few lesions on *Nicotiana glutinosa* when diluted 1 to 1,000,000, and consistent infection has occurred on both turkish tobacco and *N. glutinosa* at dilutions of 1 to 100,000. Both viruses have been recovered in a rather high concentration from leaves of tobacco dried for 7 years at room temperatures, and also have remained active when held *in vitro* at room temperatures (21° to 27° C.) for 3 years.

Protective Reaction

The physical properties, together with the identity of host range and close similarity of symptoms on most of the host plants tested, indicate that the leaf-shriveling virus is a strain of *Marmor tabaci*. Further evidence on this point has been obtained from protective tests on *Nicotiana sylvestris* of the type described by Kunkel[1] in connection with studies of the aucuba strain of tobacco mosaic.

The leaf-shriveling virus, like that of aucuba mosaic, produces necrotic primary lesions on *Nicotiana sylvestris,* but is not ordinarily systemic. However, when plants of *N. sylvestris* are first inoculated with the tobacco-mosaic virus, and inoculations are then made with the tomato virus to leaves that begin to show symptoms of tobacco-mosaic infection, either no primary lesions are produced by the tomato virus, or such lesions as do occur are few in number and are apparently confined to areas of the leaf where the tobacco virus has not become fully systemic. Such protective action of the tobacco-mosaic virus further indicates that the leaf-shriveling virus is a related strain.

[1] Kunkel, L. O. Studies on acquired immunity with tobacco and aucuba mosaics. Phytopath. 24: 437–456. 1934.

When tomato plants have been infected with the tobacco-mosaic virus for 30 days or more before inoculation with the tomato virus, there frequently is no later development of necrotic lesions or, at most, only a slight evidence of yellowing or necrosis. In tomatoes the degree of dominance of the green mottle of tobacco mosaic depends on the length of time the plants have been infected with this virus before subsequent inoculation with the leaf-shriveling virus. When tomato plants are inoculated with the latter virus within 15 days of the time the first symptoms of tobacco mosaic appear, they later develop a considerable amount of leaf necrosis. Plants first infected with the tomato virus, and then inoculated with the tobacco-mosaic virus after necrotic symptoms have appeared, show little reduction in the necrotic symptoms during later growth.

When tobacco plants are inoculated first with the tomato virus and later with tobacco mosaic (or *vice versa*) the symptoms remain those of tobacco mosaic. In all such plants the tomato virus persists in a fairly high concentration and produces abundant lesions in *Nicotiana sylvestris*. This also is true in all tomato plants, regardless of the degree of suppression of the necrotic symptoms by the tobacco-mosaic virus.

Serological Relationship

Additional evidence regarding this relationship to tobacco mosaic has been supplied by serological tests made through the kindness of F. O. Holmes and L. M. Black of the Department of Animal and Plant Pathology of the Rockefeller Institute for Medical Research. These tests have indicated that the tomato virus is serologically related to that of typical tobacco mosaic, since juices of tobacco plants infected with the tomato virus gave a characteristic precipitate when mixed with anti-serum of the tobacco virus. These tests have not necessarily eliminated the possibility that the plants carried tobacco mosaic plus another virus, but this seems unlikely in view of studies made with regard to such a possibility.

During the fall of 1940, some tomato plants inoculated with tomato virus developed numerous, small, circular spots on the inoculated leaves and on those directly above them. These spots were sharply defined and enlarged more slowly than did the diffuse, yellow areas characteristic of the virus. This suggested that we might be dealing with a combination of the ordinary green tobacco mosaic and a peculiar yellow strain. To test this, 52 inoculations were made to turkish tobacco, and 60 to Globe tomato, with fragments of tissue cut from the yellow spots. All of the inoculated tobacco plants showed the typical mottle of ordinary green mosaic. All of the tomatoes developed typical leaf necrosis, but only 6 showed the peculiar yellow spots. Twenty inoculations from green tissue of the same leaflets from which yellow tissue was taken all produced typical necrotic symptoms in tomato with two of the plants showing spotting. The same number of inoculations on tobacco produced only typical green mottling.

Another set of inoculations to tomato with necrotic tissue from lesions on leaves of *Nicotiana sylvestris* inoculated from a yellow-spotted leaf, produced typical necrosis in all of the 36 plants inoculated, but no spotting occurred. Twenty-eight tobacco plants, similarly inoculated, showed typical green mosaic.

Further subinoculations from yellow spots on the leaves of the tomato plants inoculated with spot tissue failed to reproduce the spotting, and several later subinoculations from these plants never again produced spotting. Subinoculations to tomato from 34 of the tobacco plants inoculated with tissue from yellow spots also produced typical leaf necrosis, but none of the peculiar yellow spots.

These results, together with other data and observations on the variation in symptoms produced by this virus strain, have led us to believe that the peculiar spotting resulted from environmental factors rather than from a yellow-spotting strain mixed with a green type. Had the yellow spots been the localized expression of a yellow virus, occurring in comparatively pure form, one would have expected that inoculations from this tissue would result in a decidedly more yellow type of symptom on tobacco and tomato. Further, the fact that no yellow mottle or flecking has ever been noted with this virus on tobacco supports the belief that the disease on tomatoes is caused by a single virus.

In view of all the evidence on the strain relationship between the leaf-shriveling virus of tomato and typical tobacco-mosaic virus, it appears that we are dealing with a strain of the latter virus whose severe effect on tomato foliage is its most distinctive character. Under the classification of Holmes[2] it is proposed that this virus be known as *Marmor tabaci* v. *siccans* var. nov.

Sources of Primary Infection

The question of the sources of primary infection has presented a peculiar problem in the case of the leaf-shriveling virus because of its sporadic appearance in a single greenhouse at times when no infection was known to exist on plants in other houses nor, as far as could be determined, out-of-doors.

The greenhouse in which the disease has appeared is used for tomato breeding and two crops are grown each season. The soil is usually sterilized with steam before each planting and it does not seem likely that the infection is attributable to the presence of the virus in plant debris in the soil. This is confirmed by the fact that when one crop has been severely damaged, the succeeding crop frequently has shown no evidence of the leaf-shriveling virus.

The disease has been observed only once in the field and then was found on only a few plants that apparently had been infected while growing as seedlings in the greenhouse. No wild hosts have ever been noted near the greenhouses, the location of which precludes any common occurrence of such weeds. The lack of general field infection also indicates that wild hosts are not prevalent. Furthermore, the disease has appeared in mid-winter in a year when it has not been found on the fall crop. The presence of the disease on inoculated plants might account for its appearance elsewhere in the greenhouse range, but the disease often has occurred when no inoculated material was present. Also, those working in the houses where the breeding crop is grown have had little or no contact with tomatoes in other houses, and care has been taken that they wash their hands before handling the plants.

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[2] Holmes, F. O. Handbook of phytopathogenic viruses. Burgess Publ. Co. (Minneapolis). 1939.

In several instances, the first infection from the leaf-shriveling virus has occurred on small plants within two weeks after they have been transplanted to the ground-beds. In these instances, no evidence of ordinary tobacco mosaic infection has occurred until considerably later, and an examination of the seedlings remaining after transplanting has shown no other evidence of virus infection, even when they have been held for some time. The appearance of the disease on small plants does not necessarily indicate that the virus is carried in the seed, but it often has been found that the infected plants were grown from seed of plants also affected with the leaf-shriveling virus, and this, together with the peculiarly sporadic occurrence of the disease on the small plants, has seemed to warrant a study of the seed as a source of primary infection.

Inoculations on turkish tobacco and *Nicotiana glutinosa* with sterile-water extracts obtained by soaking samples of tomato seed from which the infected plants were grown, as well as other lots of seed from plants known to be infected, have always shown a high virus concentration on the coats of seed from 1 to 4 years old. This is in line with results reported by the writers[3] in trials made with seed from plants infected with ordinary tobacco mosaic.

Planting trials have been made with seeds of various ages known to carry the leaf-shriveling virus on their surface. In most instances these trials were conducted in greenhouses well isolated from other tomato plantings. The plants were grown in sterilized soil, either in flats, or more commonly, in pots. The flats and pots also were sterilized before use.

In these planting experiments, the only evidence of seed transmission has occurred with seed planted within ten days after extraction. In one experiment (1938), 183 seedlings were grown from seed from infected tomato plants. Five of these seedlings showed mosaic symptoms in the first true leaves, and developed the necrosis typical of the leaf-shriveling virus. Two of 246 seedlings grown from the same seed, after 10 days' drying, also were similarly infected. Three of the 123 control plants showed cases of ordinary mosaic, all of which occurred some time after that on the seedlings from infected seed, and were not accompanied by necrotic symptoms. While this series was felt to be indicative of virus transmission in freshly extracted

[3] Doolittle, S. P., and F. S. Beecher. Seed transmission of tomato mosaic following the planting of freshly extracted seed. Phytopath. 27: 800–801. 1937.

seed, it was not considered conclusive, since the plants were not completely isolated from other tomato plantings.

In a later experiment (1940) tests were made in which freshly extracted seeds were grown in an isolated greenhouse at a time when there were few tomato plants in any of the houses. The seeds were planted singly in pots of sterilized soil. The planting was so arranged that pots with seed from virus-free plants were alternated in a checkerboard arrangement with those containing seed from infected fruits. The seedlings were not touched after they appeared, except that those showing virus infection were carefully removed as soon as they were noted.

In two such series a total of 5 out of 342 seedlings from seed of infected fruit developed symptoms in the first leaf, and later showed a typical necrosis. All of these seedlings were much stunted, and the necrotic symptoms developed much more slowly than in older plants inoculated mechanically.

Later trials were made with the remainder of the same seed after it had been dried for 8 days (49 plants), 20 days (51 plants) and 35 days (117 plants). The arrangement and location of the experiments were the same as in the previous trials. In these experiments all of the plants, including 196 controls, remained healthy.

In tests with 1,422 tomato plants grown from seed taken from infected fruits and aged for 30 days to 3 years, only one plant has shown evidence of the disease. This single infection was an apparent contamination, since it did not occur until after the plants had grown for over 30 days. All of the 617 controls, grown from seed of mosaic-free plants, remained healthy. Observations of over 3,000 seedlings, grown from seed of infected plants in the course of other experimental work, have indicated that, as with the ordinary tobacco-mosaic virus, aged seed is rarely if ever a source of primary infection.

These results are like those reported by the writers[4] in the case of the virus of ordinary tobacco mosaic. While this occurrence of the leaf-shriveling disease on seedlings grown from freshly extracted tomato seed is of interest, the results do not account for the greenhouse infections, since in those instances the tomato plants were grown from seed stored for 60 days to 1 year or more.

There is, perhaps, a possibility that the necrotic virus may occasionally occur in manufactured tobacco, since its effect on the tobacco plant is no more severe than that of ordinary mosaic, and would not prevent the use of the leaves for commercial purposes. This is possible, but, if so, the virus should be fairly common on tobacco, and, under such circumstances, one would expect it to occur more frequently on tomatoes in the field. At present it is difficult to account for its appearance in the greenhouse under the circumstances in which it has occurred.

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[4] See footnote 3.

SUMMARY

Tomatoes in experimental greenhouses in the vicinity of Washington, D. C., at times have been affected by a virus causing a reddish-brown necrosis of the leaflets followed by a gradual shriveling of the older foliage. There are no symptoms on the stems or fruit.

As far as determined, the physical properties and host range of this virus are the same as those of ordinary tobacco mosaic, and the symptoms are the same on tobacco and on nearly all other hosts tested. Serological tests indicate a relationship between the two viruses.

On *Nicotiana sylvestris*, where the tomato virus produces lesions like those of aucuba mosaic, the previous infection of the plants by the tobacco mosaic virus protects against infection by the virus from tomato.

Since all comparative evidence indicates that the virus causing leaf-shriveling of tomato is a strain of the tobacco mosaic virus it has been classified as *Marmor tabaci* var. *siccans* var. nov.

Seed transmission has occurred in tomato seedlings grown from freshly extracted seed of infected fruits, but no such transmission has been noted when seed was dried for more than 10 days.

The virus has appeared sporadically in the greenhouse for several years, but the sources of primary infection remain in doubt.

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THE REACTION OF CANTALOUPE STRAINS TO POWDERY MILDEW

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INTRODUCTION

Cantaloupe powdery mildew (*Erysiphe cichoracearum* DC.) first assumed epiphytotic proportions in the Imperial Valley, California, in 1925 and 1926 (1, 4). As a result of investigations by the U. S. Department of Agriculture and the California Agricultural Experiment Station, a variety was developed by 1936 that seemed highly resistant to the parasite. This variety, Powdery Mildew Resistant Cantaloupe No. 45, proved very satisfactory from the standpoint of both the grower-shipper and the consumer (2). With the advent of No. 45 into general usage the disease problem seemed to have been solved, at least temporarily. In 1938, however, a new race of *E. cichoracearum* appeared to which No. 45 and all other commercial varieties of cantaloupe were extremely suspectible (3). Since then several supposedly tolerant melon selections have become available to growers, but it is not known how these strains will behave under very severe disease conditions.

Preliminary work has indicated that in our melon[1] seed stocks there are some strains highly resistant to both races of the pathogen, but the genes for resistance are scattered through a wide range of material. Furthermore, the material was heterozygous for resistance factors and the melons were not commercially acceptable. It is, therefore, necessary to collect the genes concerned in producing immunity and combine them with factors responsible for desirable commercial qualities in the vines and fruit.

Before satisfactory progress in developing an acceptable, resistant cantaloupe variety or, even before the so-called tolerant strains can be adequately checked for ability to withstand the ravages of mildew, a rapid and precise method of gauging individual plant resistance must be devised. If such a technique were available for greenhouse use, resistance could be determined and susceptible lots eliminated before planting in the field. Further, resistant plants in a segregating population could be detected and set out in the field for observation and use in the breeding program. Such a procedure would not only dispense with land used and time consumed in growing worthless susceptible plants but also would enhance the possibility of securing immune plants with good fruit quality.

Therefore, it has been the purpose of the present investigation (1), to devise a greenhouse method of testing for mildew resistance that would be more rapid and reliable than natural field infection and, with this technique (2), ascertain the range of resistance in the breeding material at hand,

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[1] Terminology used by Wiant (10) is followed in this paper.

and (3), determine the mildew tolerance of varieties now available to commercial growers.

MATERIALS AND METHODS

In the greenhouse, plants were inoculated with a culture of race 2 of *Erysiphe cichoracearum,* described elsewhere (5), when the first true leaves had appeared. The inoculation technique and equipment used in other research (5) were employed. Disease notes were taken 16 days after inoculation. After the reaction of each plant had been noted, desirable individuals were transplanted to field plots at Torrey Pines, and Brawley, California, to determine their resistance under field conditions. Disease notes in field plantings were taken at about the time the first melons were ready for harvest.

Supposedly tolerant[2] strains were checked for resistance in the greenhouse and seed of the same lots planted in randomized and replicated field plots near Brawley in 1941. Plantings of 18 cantaloupe strains with 18 plants per strain in each of 3 blocks were made at 3 different dates. An analysis of variance (7) was made on these data.

EXPERIMENTAL RESULTS

Greenhouse Symptoms[3]

It is necessary to understand the manner in which plants, differing in ability to withstand mildew attack, respond to artificial inoculation with the fungus before the reaction of various melon strains can be adequately studied and before an arbitrary mildew-resistance rating can be devised. For these reasons a detailed description of powdery-mildew symptoms is given below:

Leaves. Macroscopically visible mycelium appeared on susceptible plants 4 to 6 days after inoculation and 7 to 10 days later mycelium covered the leaf. In the different cantaloupe strains the incubation period, mycelial development, and degree of sporulation varied, depending upon plant resistance (Fig. 1). Of these factors the incubation period seemed to be influenced least by the different host strains. The more resistant the plant, the longer the leaf remained green and vegetative.

Another type of reaction, which also seemed to be related to the resistance of the various melon strains, was a range of necrotic or chlorotic spotting, generally with mycelial development suppressed or absent (Fig. 1, C). Badly necrotic areas eventually became dry and brittle. When a great number of conidia were concentrated in a small area of a fully expanded resistant leaf during the inoculation procedure, severe necrosis often resulted in this spot.

Cotyledons. The symptoms on the cotyledons were similar to those on leaves, but in only a few plants of some strains did these structures remain entirely mildew-free. In general, they seemed more susceptible than leaves.

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[2] Throughout this paper the expressions "tolerant," "susceptible," "resistant," and the like refer to host reaction to race 2 of the powdery mildew unless otherwise stated.

[3] A preliminary report of these symptoms has already been made (6).

Macroscopically visible mycelium appeared on cotyledons of susceptible plants 1 to 2 days earlier than on the leaves. Since they were exposed before the first true leaf unfolded, this earlier appearance of mildew may have resulted from inoculum from nearby infected plants. The rate of senescence increased with the severity of infection; in many cases death resulting from senescence was difficult to distinguish from that caused by the fungus.

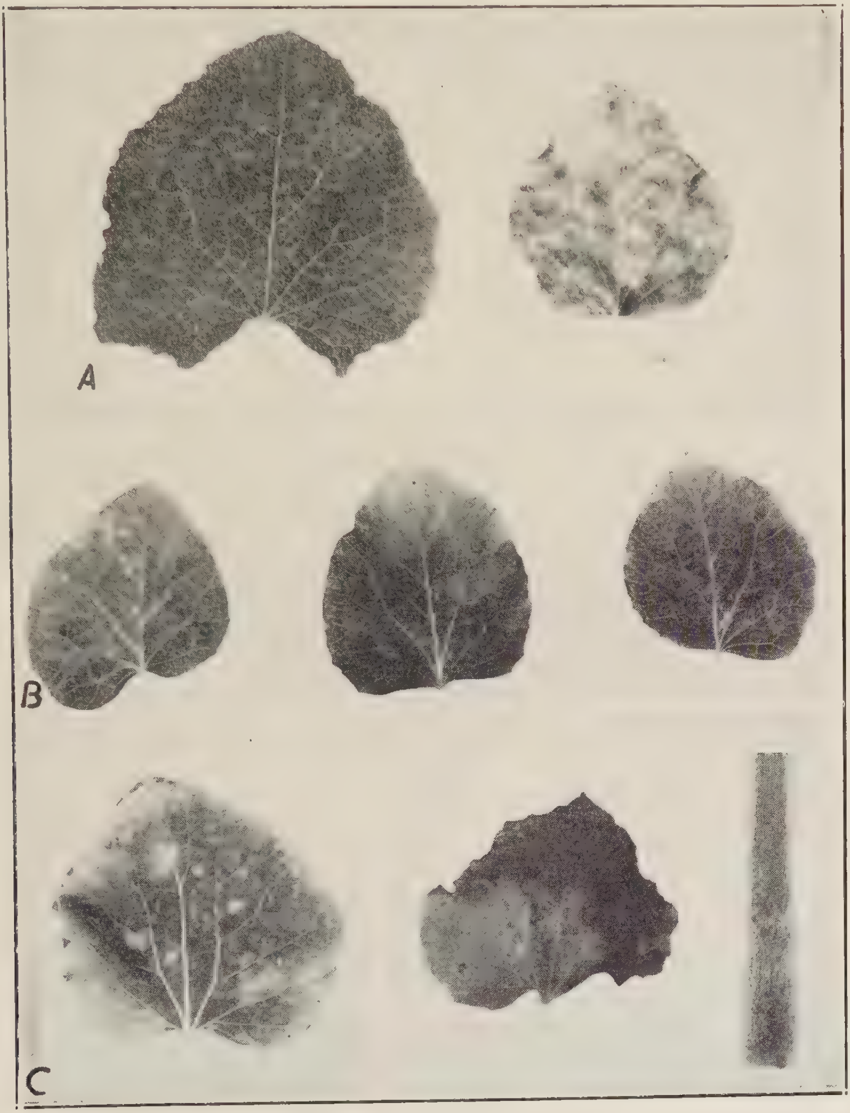

FIG. 1. Powdery mildew on cantaloupes differing in resistance. A. Highly resistant leaf compared with a susceptible leaf. B. Leaves upon which mycelial development and sporulation is suppressed. C. Leaf necrosis and stem cracking.

Stems. Visible mycelium usually appeared somewhat later on the stems of susceptible plants than on the leaves. This may not have been attributable solely to a longer incubation period, but, rather, to a less severe or delayed inoculation. The stems being vertical and shaded by the leaves would have had less surface exposed to air-borne falling spores. The degree of stem reaction was related in general to the extent of mycelial development on the leaves, except for plants of some strains in which the leaves became infected, while the stems remained free, and certain others in which the leaves were free, while the stems were mildewed. In a few cases the organism spread from the basal portion of heavily attacked cotyledons so that it entirely encircled the stem, but the fungus did not develop further from this point. No symptom exactly similar to leaf necrosis was observed on the stems, but a type of longitudinal cracking was noticed occasionally (Fig. 1, C). Frequently, stem necrosis appeared at the soil line. Since many spores were washed from the leaves and cotyledons to the soil during the watering, corrosion at this point may have resulted in some cases from repeated inoculation on resistant stems.

Rating the Amount of Mildew. For convenience in taking notes and recording results symptoms have been designated as follows:

Type 0—No mycelium evident to the naked eye.

Type 1—Only 1 to 3 small colonies developed. These rarely enlarged to more than 2 or 3 mm. diameter; seldom sporulated abundantly. In a few cases it was noticed that on plants with this grade of resistance, mycelial colonies had elongated over midrib or main veins, or sometimes concentrated at base of midrib where it joins the petiole—very slight infection.

Type 2—Little mycelium developed and few conidia formed. Colonies few, 1 to 4 mm. in diameter; confined to no particular area or there was rather general development of very diffuse mycelium giving faint grayish tinge to infected areas—slight infection.

Type 3—Mycelium sparsely covered part or all of the leaf. Sporulation somewhat suppressed—medium infection.

Type 4—The mycelium entirely covered the leaf. Sporulation abundant—severe infection.

Type A—Used in connection with the other reaction types to indicate stem cracking and necrotic or chlorotic spotting on leaves and cotyledons. No attempt made to evaluate degree of necrosis, since in most strains the symptom was mild.

FIELD REACTION

For comparison with strains tolerant to race 2, No. 45 and a highly resistant inbred were employed. Most of these strains had been mass-increased in isolated plots and, consequently, were more or less heterozygous for fruit and vine characteristic and for mildew resistance. The relative reactions of these 18 strains are shown in table 1. A partial description of these strain is as follows:

1.—No. 45, susceptible to powdery mildew race 2.

2 to 8.—Selections from No. 45, thought to possess some mildew resistance.

9.—An F_5 selection from a cross of No. 45 with a cantaloupe of unknown origin. In the greenhouse this strain has always been free of macroscopically visible mycelium but responds to artificial inoculation with the production of necrotic spots. Although the fruit is not desirable commercially, it is used here as a standard for high resistance.

10.—Powdery Mildew Resistant No. 8. This strain was thought to possess some mildew tolerance; in certain crosses seems to have contributed factors for resistance.

11.—An F_4 selection from a cross between No. 45 and No. 8.

12 to 15.—Selections from commercial D–2. This type (D–2), apparently the result of a chance cross between No. 45 and No. 8.

16.—An F_3 selection from a cross between No. 45 and D–2.

17 and 18.—Selections from Hale's Best, a commercial variety, susceptible to both races of pathogen.

TABLE 1.—*Mildew reaction of some cantaloupe strains in field plots at Brawley, where they were exposed only to natural infection*

Melon strain[a]	Average disease index[b]		
	First planting	Second planting	Third[c] planting
1	3.778	4.000[c]	4.000
2	2.167	2.463	4.000
3	2.759	3.463	4.000
4	2.037	3.037	4.000
5	3.519	3.611	4.000
6	3.278	3.463	4.000
7	2.889	3.815	4.000
8	3.463	3.352	4.000
9	0.000[c]	0.000[c]	0.000
10	3.593	3.352	4.000
11	3.685	3.741	4.000
12	2.389	2.444	4.000
13	2.981	3.241	4.000
14	2.926	3.241	4.000
15	2.833	3.074	4.000
16	3.481	3.704	4.000
17	3.352	3.667	4.000
18	1.593	2.926	4.000
Least signif. mean diff. at 5% level	0.771	0.777	....
Date of planting	12/4/40	12/23/40	1/27/41
Date of notes	5/8/41	5/21/41	6/11/41

[a] All strains except No. 9 rated 4.0 in the greenhouse test.

[b] Based upon amount of mycelium and degree of sporulation: 0—no mycelium; 1—very slight amount of mycelium; 2—slight mycelium, sporulation suppressed; 3—medium mycelium, sporulation suppressed somewhat; 4—abundant mycelium, sporulation vigorous.

[c] Not included in the statistical analysis.

Usually, early melons are more severely attacked by powdery mildew than are those of other plantings. The early-cantaloupe region is largely confined to the western part of the Imperial Valley, where the frost hazard is least and the soil is fairly light. This area also produces much early squash upon which some mildew can almost always be found throughout the winter months. The more severe infection of these early melon plantings may perhaps be attributed to the more or less constant presence of inoculum and to environment favorable for development of the parasitic complex. It will be noticed that the early plantings at Brawley (Table 1) had considerably less mildew, the difference between plantings being statistically significant. Since these plots were about 6 miles from early melons or squash, scarcity of inoculum may account in part for the lower disease incidence. There were significant differences between blocks in the second planting but they could not be accounted for on the basis of present knowledge.

In the first planting, 9 of the melon strains selected for powdery mildew

TABLE 2.—*Powdery mildew reaction of cantaloupe plants inoculated in the greenhouse and transplanted to the field where they were exposed to natural infection*

Transplanted to field plots at Torrey Pines							Transplanted to field plots at Brawley			
Greenhouse reaction[a]			Field reaction[a]				Greenhouse reaction[a]			Field reaction[a]
Plant organ	Mildew reaction	Number of plants	0	2	3	4	Plant organ	Mildew reaction	Number of plants	0
			No. plants	*No. plants*	*No. plants*	*No. plants*				*No. plants*
Leaf	0	49	46	1	2	0	Leaf	0	43	43
	0A	73	70	0	3	0		0A	27	27
	1	8	7	0	0	1		1	13	13
	1A	11	11	0	0	0		1A	7	7
	2	6	5	0	0	1		2	21	21
	2A	6	4	0	1	1		2A	4	4
	3	9	6	0	1	2		3	4	4
	4	4	2	0	0	2		......	......	......
Stem	0	66	63	1	1	1	Stem	0	66	66
	0A	32	29	1	2	0		0A	9	9
	1	13	13	0	0	0		1	10	10
	1A	9	9	0	0	0		2	6	6
	2	14	12	0	1	1		3	22	22
	2A	5	5	0	0	0		4	11	11
	3	17	15	0	0	2		......	......	......
	3A	2	2	0	0	0		......	......	......
	4	8	4	0	3	1		......	......	......

[a] Only those disease index groups in which plants occurred are included in the table. Disease index based on amount of mycelium, degree of sporulation, and presence or absence of necrosis or chlorosis: 0—no mycelium; 1—very slight amount of mycelium; 2—slight mycelium, sporulation usually suppressed; 3—medium mycelium, sporulation usually somewhat suppressed; 4—abundant mycelium, sporulation vigorous; A—presence of necrosis or chlorosis. Notes on necrosis were not taken in the field because the symptom was difficult to separate from that caused by agents other than powdery mildew.

tolerance (disregarding number 9) were significantly less susceptible than number 1, but none of them approached the high resistance found in number 9. Except for numbers 8, 9, and 10, the second planting was more severely attacked than the first. In this planting only 5 of the tolerant lines appeared to be significantly more resistant than number 1. In the last planting, all of the strains, except the highly resistant number 9 were very heavily infected.

Comparison of Greenhouse Inoculation and Natural Field Infection

All of the melon strains included in the field plantings (Table 1) have been repeatedly tested in the greenhouse, where all, except number 9, have developed a 4 reaction on the leaves, stems, and cotyledons within 16 days after inoculation. From these data it appears that certain mildew-tolerant strains may be comparatively disease-free when the inoculum is scant or environmental factors are unfavorable for the disease, but under epiphytotic conditions they are no better than commercial No. 45. Greenhouse tests lend support to this supposition.

A great many other strains were subjected to greenhouse inoculation. From these, promising individual plants were selected and transplanted to field plots at Torrey Pines or at Brawley, where observations of field resistance were made. Records for a representative number of these selected plants in the 1940 and 1941 plantings are shown in table 2. A large majority were completely free of mildew at the time the first melons were picked.

In a few cases leaves or stems of some plants appeared to be more severely infected in the field than in the greenhouse, indicating that the greenhouse method was not always so effective in producing severe disease symptoms as

TABLE 3.—*Powdery mildew reaction of individual cantaloupe plants that appeared to be more resistant in the greenhouse than in the field*

Plant number	Greenhouse reaction[a]			Field reaction	
	Leaves	Cotyledons	Stems	Leaves	Stems
8–2M–2	0A	4A	0	2	2
16046–1	0A	4	3	2	0
16111–1	0	3	0A	3	3
16111–2	0	4	0A	3	3
16111–3	0	4	0A	2	2
29082–3	1	4	2	4	3
29442–10	2	2	2	4	4
29442–12	3	4	4	4	3
29442–13	0A	0	0	2	3
29443–3	2A	4	3	4	4
29443–5	4	4	3	4	4
29443–6	3	3A	0	4	4
29443–14	3	3	4	3	3
29443–16	2A	3A	4	3	3

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[a] Disease index based on amount of mycelium, degree of sporulation and presence or absence of necrosis or chlorosis: 0—no mycelium; 1—very slight amount of mycelium; 2—slight mycelium, sporulation usually suppressed; 3—medium mycelium, sporulation usually somewhat suppressed; 4—abundant mycelium, sporulation vigorous; A—presence of necrosis or chlorosis.

were natural field conditions. Of particular interest in this connection are plants that had a 0 reaction on leaves or stems in the greenhouse, yet showed some mildew in the field, since these individuals probably would be considered desirable as resistant parents. Table 3 shows the powdery mildew reaction of the individual plants whose leaves or stems appeared more resistant in the greenhouse than in the field. In the greenhouse all of these plants manifested symptoms of mildew either on the leaves or the stems, although not necessarily on both organs of the same plant. For example, plant 16111–1 showed a 0 reaction on the leaves, but a 0A reaction on the stems in the greenhouse, indicating a slight degree of infection; whereas the field reaction was 3 for both leaves and stems. However, at no time during the two years this problem has been under investigation has a plant with a 0 reaction on both leaves and stems in the greenhouse developed any mildew in the field. In only a few cases have plants, free of macroscopically visible mycelium on leaves or stems, but showing some necrosis or chlorosis, had mildew when transplanted to field plots. On the other hand, quite a few plants rated 1, 2, or 3 in the greenhouse have become more severely attacked in the field.

From the above data it is evident that (1) there exists in our breeding stock strains that are highly resistant to powdery mildew, both in the greenhouse and in the field; (2) the greenhouse technique may be used effectively in selecting very highly resistant plants; (3) it is not so reliable in evaluating different degrees of field tolerance; (4) the reaction of both leaves and stems must be considered in making selections.

DISCUSSION

Repeated tests have illustrated that a uniform and severe disease infection always may be obtained in the greenhouse, whereas, in the field, host reaction to mildew is not the same throughout the year. Summer and fall plantings in the Imperial Valley often are made to increase seed of desirable lines. Observations over several years have shown that these plantings almost always remain mildew-free. Environmental factors may account for this lack of infection. Yarwood (11) suggested that high temperatures, combined with low humidity, may be injurious to the powdery mildews. His studies were made in part on *Erysiphe cichoracearum* from sunflower, and may not be exactly comparable to the biologic race on cantaloupe. However, the field observations mentioned above indicate that the cantaloupe race of the pathogen may be sensitive to high temperature and low humidity, which, if true, would reduce inoculum during the summer. Light intensity and plant resistance at different ages also should not be excluded from consideration as factors possibly influencing the amount of mildew.

In the early plantings, where mildew is often severe, environment may possibly tend to reduce the resistance of lines that at other times of the year are comparatively disease-free. Although the effect is reversed, a situation perhaps comparable is found in the "type B" resistance of cabbage to yellows (8, 9); there, high temperature brings about disease development in certain varieties that, during cool weather, seldom show severe yellows symptoms.

Plants with some necrosis or a small amount of mycelium on leaves or stems under greenhouse conditions cannot always be relied upon to be highly resistant in the field. However, many strains, having leaves with necrotic spots but no mycelium visible to the naked eye, did not become infected in the field plantings. From the results of this investigation it would seem that the greenhouse method of eliminating susceptible lines is more efficient and reliable than field trials performed throughout the year, provided all macroscopic leaf and stem symptoms be taken into account.

SUMMARY

Cantaloupe plants, differing in resistance to powdery mildew, were artificially inoculated in the greenhouse with race 2 of *Erysiphe cichoracearum*, and the range of symptoms described. Five classes of symptoms based on mycelial development and degree of sporulation were employed in rating the susceptibility of these plants, 0 to 4 indicating the range from absence of visible mildew growth to abundant, vigorously sporulating mycelium. Leaves and cotyledons of some plants also responded with the production of faint yellow to definitely necrotic spots, usually with mycelium restricted or absent. No symptoms exactly similar to leaf or cotyledon spotting appeared on stems but a longitudinal cracking was noticed occasionally. Leaf, cotyledon, and stem reaction differed greatly between the various melon strains; and, on individual plants, these organs were seldom attacked with equal severity, except in the susceptible strains, where they were entirely covered with mildew. Other conditions being constant, the symptoms produced on leaves, cotyledons, or stems appeared to be a function of the host genotype. In general, cotyledons seemed more susceptible than leaves or stems.

Duplicate plantings of 18 cantaloupe strains were made in the greenhouse and at 3 different dates in field plots. These 18 lines comprised selections from Hale's Best (susceptible to both mildew races), Powdery Mildew Resistant Cantaloupe No. 45 (resistant to race 1 but not to race 2), a strain highly resistant to both races of the pathogen, and several strains thought to be tolerant to both mildew forms. In the greenhouse all strains, except the one highly resistant to both fungus races, had a 4 reaction on leaves, stems and cotyledons. Mildew severity increased in each successive field planting, all the plants in the third planting, except the highly resistant strain, having a 4 reaction. The majority of the tolerant strains were significantly better than No. 45 in the first planting, and several were superior in the second.

Data from the reaction of individual plants in the greenhouse and of these same plants after being set out in the field showed that the greenhouse method is much more reliable than field trials as a tool for selecting highly

resistant plants, provided all macroscopic leaf and stem symptoms are taken into account.

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CROWN GALL ON SPECIES OF TAXACEAE, TAXODIACEAE, AND PINACEAE, AS DETERMINED BY ARTIFICIAL INOCULATIONS[1]

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Results of inoculation of species of Pinaceae and Taxaceae with *Phytomonas tumefaciens* (Smith and Townsend) Bergey *et al.* have been reported by the writer on *Sequoia* spp. (1), on *Araucaria bidwillii* (2), *Libocedrus decurrens* (3), *Taxus baccata* var. *erecta* (4), and on species of Cupressaceae (5). Results of further studies are herein reported.

The trees for these tests were secured from local nurseries; they were established in 5-gal. containers and grown in a lath house. Trees of *Sequoia* spp. also were grown in the open.

Inoculations were made by the puncture method, with a sterilized steel needle and pure cultures of the crown-gall organism. Control punctures were made on all the different species under experimentation; and in all cases the wounds healed normally.

TABLE 1.—*Summary of inoculations with Phytomonas tumefaciens on species of conifers*

Species inoculated	Number of inoculations	Number of galls	Diameter range (mm.)
	Taxaceae		
Cephalotaxus fortunei	75	0	......
Podocarpus elongata	145	3	15–22
Podocarpus macrophylla	15	0	......
Taxus baccata var. *erecta*	80	50	10–20
Taxus brevifolia	15	4	5–15
Taxus cuspidata	65	0	......
Taxus media	45	4	2– 4
Torreya californica	15	7	10–30
	Taxodiaceae		
Cryptomeria japonica	60	0	......
Cryptomeria japonica var. *elegans*	70	0	......
Cunninghamia lanceolata	40	9	6–40
Sciadopitys verticillata	65	5	2– 5
Sequoia gigantea	35	6	15–25
Sequoia sempervirens	60	4	2–60
Taxodium distichum	50	0	......
Taxodium mucronatum	220	0	......
	Pinaceae		
Abies cephalonica	66	2	5–10
Abies concolor	37	5	5–20
Abies firma	81	1	10
Abies holophylla	21	2	5–25
Abies nephrolepis	15	0	......

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[1] Paper No. 462, University of California Citrus Experiment Station, Riverside, California.

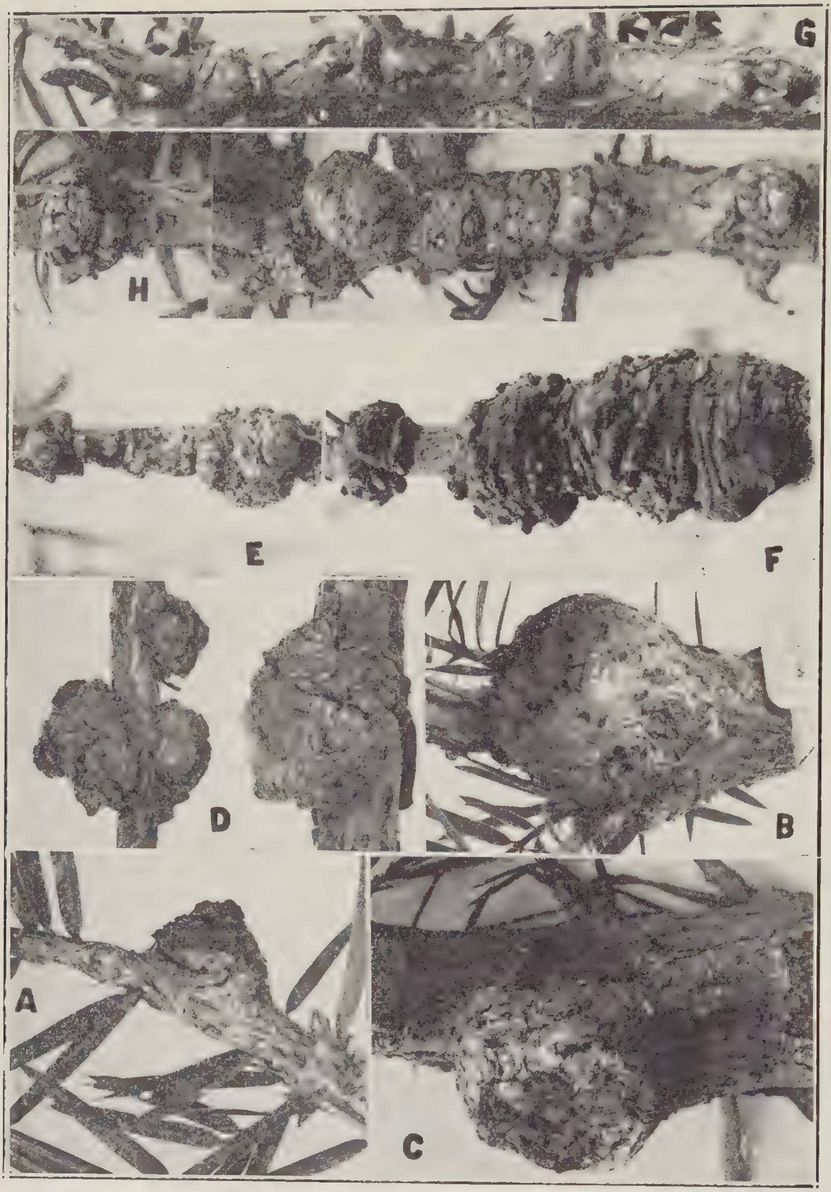

FIG. 1. Artificial galls produced on species of Taxaceae by inoculation with *Phytomonas tumefaciens* isolated from *Salix* sp. for all galls except those on *Taxus baccata* var. *erecta*, for which cultures were isolated from *Prunus persica*. A to C. Galls on *Torreya californica:* A, 15 months after inoculation; B, after 22 months; C, after 29 months. D. Galls on *Taxus baccata* var. *erecta*, after 60 months (galls long inactive). E and F. Galls on *Podocarpus elongata:* E, 11 months after inoculation, showing smooth, spherical form of early stages; F, after 40 months, showing irregular form and small roundish projections of later development. G and H. Galls on *Taxus brevifolia:* G, 30 months after inoculation; H, after 45 months; note increase in size of certain galls but slight change or no change in others.

INOCULATION OF SPECIES OF TAXACEAE

Species of Taxaceae tested for crown gall by artificial inoculations were as follows: *Cephalotaxus fortunei* Hook., *Podocarpus elongata* L'Her., *P. mocrophylla* Don, *Taxus baccata* var. *erecta* Loud., *T. brevifolia* Nutt., *T. cuspidata* Sieb. and Zucc., *T. media* Rehd., and *Torreya californica* Torr. The small trees of these species were inoculated at frequent intervals during the year. The cultures used on *Taxus baccata* var. *erecta* were from *Prunus persica* L. All other cultures were from *Salix* sp. Results of the inoculations are summarized in table 1.

In these tests, the artificially induced galls (Fig. 1) were not always entirely typical of crown gall as it appears on other hosts. Inoculation of *Podocarpus elongata* caused typical spherical overgrowths in the early stages of development (Fig. 1, E); but in later stages these growths became more irregular in form, and small roundish projections developed (Fig. 1, F). The results presented in table 1 suggest that *P. elongata* is strongly resistant.

Torreya californica was readily infected. The galls (Fig. 1, A to C) were slow in appearing, but, once started, their development was continuous and fairly rapid. In their early stages, some of the overgrowths were smooth (Fig. 1, B) and some were rough (Fig. 1, A). In later stages, however, galls on this host generally became rough, much like the overgrowth shown in figure 1, C.

The galls on *Taxus brevifolia* (Fig. 1, G and H) and on *T. baccata* var. *erecta* (Fig. 1, D) grew slowly, but were more typical of crown galls than those on *Podocarpus*. Some of the galls on *T. brevifolia* are still growing; others are inactive and have made but slight growth in the 2 years since inoculation. This host has, itself, made but slight growth during this time. Galls on *T. baccata* reached their maximum size, and have long been desiccated and dead.

The results of the inoculations on *Taxus cuspidata* were inconclusive. Small galls appeared on *T. media,* however, which is a hybrid between *T. cuspidata* and *T. baccata.* All the tests on *Cephalotaxus fortunei* were negative. Results of previous tests on this species were also reported as negative (4). It appears that *C. fortunei* may be resistant.

INOCULATION OF SPECIES OF TAXODIACEAE

The following species of Taxodiaceae, an important family of the conifers, were inoculated in these tests: *Cryptomeria japonica* Don, *C. japonica* var. *elegans* Masters, *Cunninghamia lanceolata* Hook., *Sciadopitys verticillata* Sieb. and Zucc., *Sequoia gigantea* DC., *S. sempervirens* Endl., *Taxodium distichum* Rich., and *T. mucronatum* Ten. Results of the inoculations are summarized in table 1.

Cryptomeria japonica, C. japonica var. *elegans, Taxodium distichum,* and *T. mucronatum* did not respond to inoculation. Galls were readily produced on *Cunninghamia lanceolata* (Fig. 2, D), *Sequoia gigantea* (Fig. 2, C), *S. sempervirens* (Fig. 2, A and B), and on *Sciadopitys verticillata*. The galls were especially well developed on *Cunninghamia lanceolata,* and this species was apparently extremely susceptible. The susceptibility of *Sequoia* spp. to crown gall was previously reported (1) and is confirmed by the results of inoculations in these tests. The galls on *Sciadopitys* were small but typical. This host, under conditions at Riverside, California, made slow growth, and the resultant galls were small knobs 2 to 3 mm. in diameter.

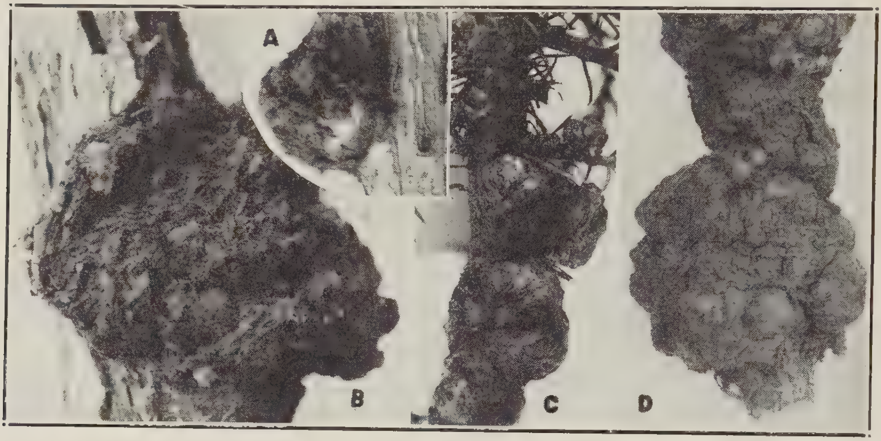

FIG. 2. Artificial galls produced on species of Taxodiaceae by inoculation with the crown-gall organism isolated from *Salix* sp. A and B. Gall on *Sequoia sempervirens:* A, 24 months after inoculation; B, after 30 months. C. Three galls on *S. gigantea,* after 7 months. D. Galls on *Cunninghamia lanceolata,* after 33 months.

INOCULATION OF SPECIES OF PINACEAE

The Pinaceae tested for crown gall by artificial inoculations included species of *Pinus, Picea, Pseudotsuga, Cedrus, Larix,* and *Abies*. No galls were obtained on the following species of *Pinus: P. canariensis* C. Smith, *P. cembroides* Zucc., *P. coulteri* Don, *P. halepensis* Mill., *P. muricata* Don, *P. pinea* L., and *P. sylvestris* L. Results on *Picea koyamai* Shiras (SPI 97948), *P. pungens* Engelm., *Pseudotsuga taxifolia* Brit., *Ps. macrocarpa* Mayr., *Cedrus deodara* Loud., *Larix decidua* Mill. (*L. europaea* DC.), and on *L. kaempferi* Sarg. (*L. leptolepis* Murr.) also were negative.

Those species of *Abies* tested showed, with the exception of *A. nephrolepis* Maxim. a definite response to artificial inoculations (Fig. 3). Inoculations were necessarily made on wood several years old. The growth of these hosts was slow, and conditions were thus unfavorable for gall formation. But, though few galls were produced from a relatively large number of inoculations, these galls were typical. Results of the tests are given in table 1.

The gall on *Abies concolor* Lindl. and Gord. (Fig. 3, E) showed old tissue still attached but being pushed off by the formation of new gall tissue. The galls (Fig. 3, C and D) were well developed and more typical. Galls on *A. holophylla* Maxim. (Fig. 3, A and B) were globose and apparently attached to the host by a small area of tissue. The galls produced on *A. firma* Sieb. and Zucc. (Fig. 3, F) and on *A. cephalonica* Loud. were on trees that were kept under a moist chamber in the lathhouse during and for some time after inoculation.

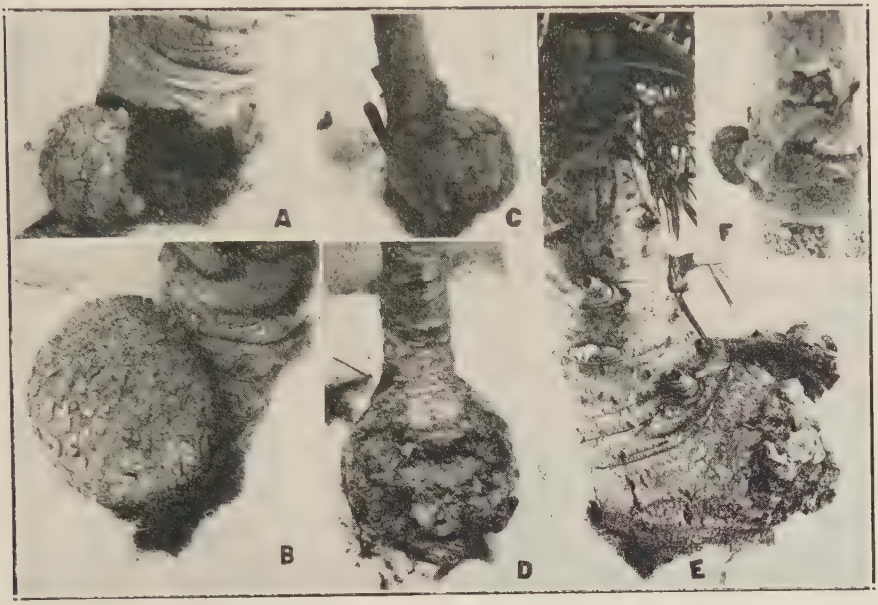

FIG. 3. Galls artificially induced on *Abies* spp. (Pinaceae) by inoculation with the crown-gall organism isolated from *Salix* sp. A and B. Gall on *Abies holophylla* (SPI 90649): A, 18 months after inoculation; B, after 52 months. C to E. Galls on *A. concolor:* C, 30 months after inoculation; D, after 65 months; E, after 60 months. F. Gall on *A. firma,* after 11 months.

SUMMARY

Inoculations with *Phytomonas tumefaciens* (Smith and Townsend) Bergey *et al.* were made on different species of conifers. Galls developed on the following species: (Taxaceae) *Podocarpus elongata, Taxus baccata* var. *erecta, T. brevifolia, T. media, Torreya californica;* (Taxodiaceae) *Cunninghamia lanceolata, Sequoia gigantea, S. sempervirens, Sciadopitys verticillata;* (Pinaceae) *Abies cephalonica, A. concolor, A. firma,* and *A. holophylla.*

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2. ————. Crown gall on *Araucaria bidwillii.* Phytopath. 26: 400–401. 1936.
3. ————. Crown gall on incense cedar, *Libocedrus decurrens.* Phytopath. 27: 844–849. 1937.
4. ————. Crown gall on *Taxus baccata.* Phytopath. 28: 153–155. 1938.
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FUSARIUM YELLOWS OF BEANS

JAMES B. KENDRICK AND WILLIAM C. SNYDER

(Accepted for publication March 9, 1942)

INTRODUCTION

In 1929, Harter[1] reported a vascular fusarium disease of field beans (*Phaseolus vulgaris* L.) in the Sacramento Valley, California, characterized by stunting of the plants, yellowing and dropping of the leaves, and almost complete vascular invasion by the fungus. The writers observed a similar disease in the same locality in 1929 and again in 1933. In 1934, Kendrick[2] reported the disease as fusarium yellows of beans and showed that it was seed-transmitted. A general survey of the district showed it to be confined to a few fields in a restricted area. These fields have been planted to other crops, and no additional infested areas have been observed in this district.

The disease was not observed again until the summer of 1940, when it was found in 2 large plantings of pink beans in the upper Sacramento Valley, California. One field of approximately 50 acres showed an estimated damage of 50 per cent, while the other showed only single isolated diseased plants. The severity of the disease and information secured from the grower indicated that it had been present in the severely diseased field for several years. In 1941, isolated diseased plants were observed in another field in this same district.

The presence of isolated infected plants in the one field in 1940 and in another in 1941 indicated introduction of the disease by seed-borne fungus spores. A rather careful examination of these two fields failed to show other than single diseased plants, usually in widely separated areas.

Repeated isolations from discolored vascular tissue of diseased plants consistently yielded the same fungus, which, in culture, resembled the cowpea (*Vigna sinensis* Endl.) wilt organism, *F. oxysporum* f. *tracheiphilum* (E. F. S.) Sny. and Hans.

SYMPTOMS

The disease first shows as a slight fading from green to yellow of the lower leaves on one side of the plant. This change from green to yellow progresses rapidly from the lower leaves upward and is in most cases much more pronounced on one side of the plant. The bright yellow leaves later fall from the plant, while the normal green to pale-green ones on the opposite side remain attached. In the later stages of the disease many plants are entirely killed, while others may retain a few sickly leaves on one side of the plant and a weak terminal growth until maturity.

The vascular system shows a dark-brown discoloration extending into the main stem, lateral branches, petioles, and peduncles. The vascular browning is typical of that occurring in the fusarium wilt of cowpeas.

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[1] Harter, L. L. A Fusarium disease of beans. (Abstract) Phytopath. **19**: 84. 1929.

[2] Kendrick, James B. Seed transmission of Fusarium yellows of beans. (Abstract) Phytopath. **24**: 1139. 1934.

PATHOGENICITY

Because of the similarity of symptoms of bean yellows and those of cowpea fusarium wilt (*Fusarium oxysporum* f. *tracheiphilum*) reciprocal inoculations were conducted along with the pathogenicity tests. For these tests, pure cultures of the bean yellows Fusarium and that obtained from cowpea-wilt-infected plants were grown on steam-sterilized oats, thoroughly mixed with steam-sterilized soil. In all trials, noninoculated steam-sterilized soil was used for controls. The soil was placed in 6-inch pots and the seed planted immediately. All pots were held in a greenhouse where the temperature was maintained at 70° F. or above. In these studies 2 species of beans, pink and red Mexican (*Phaseolus vulgaris*), and Henderson's bush Lima and Wilbur Lima (*Phaseolus limensis* Macf. var. *limenanus*, Bailey), and blackeye cowpea (*Vigna sinensis*) were used.

On November 3, 1929, 8 isolates of cowpea fusarium wilt and 6 isolates of Fusarium from bean yellows were separately inoculated into sterilized soil. Of the 48 pots of cowpea-wilt-inoculated soil, 16 were planted with blackeye cowpeas, 16 with pink beans, and 16 with Henderson's bush Lima beans. Of the 60 pots inoculated with Fusarium from bean yellows, 12 were planted with blackeye cowpeas, 12 with pink beans, 12 with red Mexican beans, and 12 with Henderson's bush Lima beans. On January 21, 1930, final records were taken on diseased and healthy plants. Plants showing no obvious leaf symptoms were pulled and examined for vascular invasion. Those plants showing vascular invasion and no external plant symptoms were cultured to determine the cause of the vascular discoloration (Table 1).

TABLE 1.—*Summary of cowpea fusarium wilt and bean yellows Fusarium cross-inoculation trials of Nov. 8, 1929, to Jan. 21, 1930*

Source of culture used	Blackeye-cowpea		Pink beans		Red Mexican beans		Henderson's bush Lima beans	
	Number of plants	Percentage diseased	Number of plants	Percentage diseased	Number of plants	Percentage diseased	Number of plants	Percentage diseased
Cowpea wilt	193	24.9	225	0.0	....	....	184	0
Bean yellows	211	0.0	231	14.2	238	25.8	206	0
Control ...	121	0.0	121	0.0	131	0.0	146	0

The above table shows that the cowpea-fusarium-wilt fungus did not attack either of the bean varieties, and the Fusarium causing bean yellows did not attack either blackeye cowpeas or Henderson's bush Lima beans.

On February 12, 1934, a similar series of greenhouse experimental plantings were made in which 3 isolates of cowpea fusarium wilt and 3 of Fusarium from bean yellows were used. In this series, blackeye cowpea, pink beans, and Wilbur Lima beans were used. On March 19, 1934, final records were taken and the summarized results are presented in table 2.

TABLE 2.—*Summary of cowpea fusarium wilt and bean yellows fusarium inoculation trials of February 12 to March 19, 1934*

Source of fungus used	Blackeye cowpea		Pink beans		Wilbur Lima beans	
	Number of plants	Percentage diseased	Number of plants	Percentage diseased	Number of plants	Percentage diseased
Cowpea	78	82.0	150	0.0	94	0
Cowpea	58	96.5	95	0.0	67	0
Cowpea	54	74.1	91	0.0	77	0
Pink bean	107	0.0	150	78.0	133	0
Pink bean	98	0.0	79	64.5	56	0
Pink bean	90	0.0	123	88.5	96	0
Controls	115	0.0	107	0.0	98	0

Table 2 shows a much higher percentage of infection than does table 1. This probably was due to the higher average greenhouse temperature and better light conditions prevalent during these tests. It further shows that the bean fusarium does not attack cowpea, the cowpea fusarium does not attack beans, and that Lima beans resist the attack of both organisms.

In the summer of 1929, 17 varieties of beans, 3 of Lima beans, and 3 of soybeans (*Soja max* Piper) were planted in a field plot naturally infested with the cowpea fusarium in Stanislaus County, California. The plants were observed throughout the summer for evidence of disease and no disease occurred. Susceptible cowpea selections planted in the same plot showed a high percentage of wilted plants.

Again in 1930, 10 varieties of beans, 3 of Lima beans, and 3 of soybeans were included in a cowpea-wilt experimental test on heavily infested soil in the same county. A careful check throughout the season failed to reveal any evidence of wilt on the bean, Lima bean, or soybean varieties, whereas the susceptible cowpea varieties were 100 per cent killed by wilt.

In 1940, a field of approximately 50 acres of pink beans showed more than 50 per cent severely diseased plants from fusarium yellows. This same field was planted to Wilbur Lima beans in 1941, and there was no evidence of the disease in any of the plants.

Thus, extensive greenhouse inoculation tests, field experiments, and field observations show that *Fusarium oxysporum* f. *tracheiphilum*, which causes cowpea (*V. sinensis*) wilt, does not attack soybean (*S. max*), contrary to statements found in literature,[3] nor are common beans (*P. vulgaris*) or Lima beans (*P. limensis* var. *limenanus*) affected by the cowpea Fusarium. Furthermore, these data show that the bean yellows Fusarium, which attacks varieties of *P. vulgaris*, does not affect the varieties of cowpea, soybean, or Lima bean reported upon here. These facts are in line with the recognized tendency of the *formae* of *F. oxysporum* to be rather narrowly specialized, usually to single genera. They show that not only are the vascular fusarium wilts of *V. sinensis* and *P. vulgaris* caused by separate and distinct physiologic forms of *F. oxysporum,* but, also, they suggest that the vascular fusarium disease of *S. max* may be caused by still another and distinct form.

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[3] Cromwell, R. O. Fusarium blight, or wilt disease, of the soybean. Jour. Agr. Res. [U.S.] 8: 421–440. 1917.

TAXONOMY

Single-spore cultures of the fungus recovered from tissue platings of the discolored vascular elements of diseased bean (*Phaseolus vulgaris*) plants proved to be morphologically in agreement with *Fusarium oxysporum* Schl., as emended by Snyder and Hansen.[4]

Since a form of *Fusarium oxysporum*, pathogenic on bean (*P. vulgaris*), has not been described heretofore, and, since data given herein indicate that the form from bean is a specialized pathogen of this host, it is proposed that this pathogen be distinguished from other forms of *F. oxysporum* by the creation of a new form name as follows:

Fusarium oxysporum f. **phaseoli**, n. f. A vascular parasite of *Phaseolus vulgaris* causing Fusarium yellows of beans.

CONTROL

Kendrick,[2] in 1934, showed that the *Fusarium* causing bean yellows is transmitted with the seed, probably by spores of the fungus adhering to the seed coat. He further showed that dusting the seed with 8 oz. of Semesan or 4 oz. of Ceresan per 100 lb. of seed completely eliminated the disease in greenhouse tests. Since the causal fungus will persist in the soil for long periods of time, it is recommended that seed from a suspected diseased area be dusted with Semesan or Ceresan before being planted in a disease-free area. Fields infected with the bean yellows fungus can be safely planted to Lima beans, soybeans, or cowpeas.

SUMMARY

A vascular fusarium disease of field beans was first observed in the Sacramento Valley, California, in 1929 and again in 1933. Due to the absence of bean plantings in the infested area, the disease was not observed again until 1940, when it was observed in another section of the valley.

The disease is characterized by a gradual yellowing of the leaves from the lower leaves upward, dwarfing of the plants, and eventual dropping of the leaves, and death of the plant in many cases. The vascular system of the stem and leaf petioles shows a dark brown discoloration.

A *Fusarium,* which in culture resembled the cowpea wilt organism was consistently isolated from diseased bean tissue.

Inoculation studies showed the bean *Fusarium* to be pathogenic to varieties of common beans (*Phaseolus vulgaris*) but not to Lima beans (*P. limensis* var. *limenanus*), cowpea (*Vigna sinensis*), or soybeans (*Soja max*).

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[4] Snyder, W. C., and H. N. Hansen. The species concept in *Fusarium*. Amer. Jour. Bot. 27: 64–67. 1940.

Since field, greenhouse, and cultural studies have shown that bean yellows is caused by an apparently undescribed form of *Fusarium*, it is herein designated as *F. oxysporum* f. **phaseoli** n. f.

The causal *Fusarium* may be transmitted with the seed, and infection can be controlled by seed treatment with Semesan or Ceresan.

Lima beans, cowpeas, and soybeans can be safely planted in bean-yellows infested soil.

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EFFECTIVE METHODS OF INOCULATING SEED BARLEY WITH COVERED SMUT (USTILAGO HORDEI)[1]

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INTRODUCTION

Covered smut of barley, *Ustilago hordei* Lagerh., causes considerable loss, much of which can be avoided by growing smut-resistant varieties. Progress in this direction, however, has long been hampered by the difficulty in getting high percentages of smutted heads in susceptible varieties through artificial inoculation of the seed. Since Jensen's (8) report in 1888, it has been repeatedly observed that when seed from a smutted crop is threshed, stored, and then sown without treatment, high percentages of covered smut frequently occur. Paradoxically, however, when clean seed is artificially inoculated by superficially blackening it with millions of spores, only low percentages of smutted heads usually occur (2, 3, 15, 16). This has been difficult to understand in view of the belief that spores of covered smut remain enclosed in the smutted heads until threshing, when the smutted heads are disintegrated and spores come in contact with the surface of seed. There would seem to be little important difference between this method of inoculation and the artificial one of shaking or rolling seeds in smut spores.

In an attempt to clarify this problem, the senior writer (15) studied inoculation as it occurs in the usual culture of barley. It was found that spores are released from smutted heads in the field from heading to threshing, as well as during threshing. Many spores and extensive ramifications of mycelium from germinated spores were found beneath the hulls of naturally inoculated seed obtained from different parts of the United States. This subhull inoculum, moreover, proved especially effective in infection. It also has been observed repeatedly in earlier investigations that the blackening of seed with smut spores is far more effective in smut production if the hulls are first removed (2, 4, 9, 16). It is evident, therefore, that the hulls interfere with infection when they come between the inoculum and caryopsis of the kernel. Manual dehulling of the seed is too slow and tedious for practical use, and no satisfactory mechanical or chemical means of dehulling has been available (9). On the basis of these facts the writers devised and studied several new methods of seed inoculation. Some of the new methods have given excellent results, year after year, under a wide variety of climatic conditions. As a result, the study of physiologic races in *Ustilago hordei* (13) and breeding for resistance against the races that have been isolated are now going forward.

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[1] Cooperative investigation of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, the New York (Cornell) Agricultural Experiment Station, and the Idaho Agricultural Experiment Station. Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper No. 191.

[2] Pathologist and Associate Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

EARLIER INVESTIGATIONS

Tapke (15) has recently reviewed the earlier studies on natural and artificial inoculation of seed barley with covered smut.

METHODS AND MATERIALS

It has been shown in recent years that the baneful effects of a poor method of seed inoculation may be largely or entirely offset by growing the plants under greenhouse conditions after emergence (14). For this reason the tests in the present study were conducted under field conditions.

The new seed-inoculation methods described herein were patterned after Haarring's (5) successful "evacuation" method for inoculating oats with loose smut (*Ustilago avenae*). In this method 1 g. of spores and 250 g. of oats are added to 1000 cc. of a special nutrient solution. The whole is stirred and evacuated for 20 minutes by suction from a water jet and the inoculated seed is spread to dry for 24 hours. The seed is then placed on moist filter paper in a chamber at 22–25° C. for 20 hours. Finally, it is dried and used at once, or stored for later use. By this method the smut spores are placed beneath the hulls close to the point of attack, as in the natural inoculation of seed. Haarring's method, while readily adaptable to a few large batches of grain, appeared too complicated for studies of physiologic races of *Ustilago hordei* involving the inoculation of several thousand samples of seed. Many simplified modifications, therefore, were tested in extensive preliminary trials and two promising methods finally were chosen for further study. These are referred to herein as the "spore-suspension" and "vacuum" methods. In the former, described previously (11), machine-threshed seed is placed in vials and covered for 15 minutes with a spore suspension made by adding 1 g. of spore dust to 1000 cc. of water.[3] During the first half-minute of this period the vials are vigorously shaken to wash spores beneath the hulls. The suspension is then decanted and the vials are inverted on clean pieces of blotting paper to absorb all free water. Next, the vials of moistened inoculated seed are packed in a tightly covered tin box, floored with a moistened blotter to maintain high humidity and promote spore germination. After 16 to 20 hours at 20° C., the vials are removed from the box, the seed is transferred to small envelopes, crimped to remain wide-open, and left for 3 or 4 days or until the seed is thoroughly dry. It is then ready to sow or to store for later use. The spore-suspension method, as thus applied, therefore, eliminates the nutrient solution,[4] the vacuum treatment, and the 24-hour preliminary drying period of Haarring's method (5).

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[3] Preparation of the suspension with spores from hard, dried heads of covered smut may be facilitated by first cutting the smutted heads into small pieces with scissors. Water, sufficient to cover the pieces is then added, and the smut is soaked until it is soft. The smut masses are then mashed and water is added to make up the desired amount of suspension. The suspension is then poured into a clean container through a double layer of cheesecloth to strain out remnants of the smutted heads.

[4] Western (17) used aqueous and nutrient suspensions of oat-smut spores to inoculate

Procedure in the vacuum method is the same as that in the spore-suspension method, except that after the seed is put in vials and covered with the spore suspension, the vials are placed in a special jar and subjected to 30 inches of vacuum for 15 minutes. The spore suspension is then decanted and the seed is incubated for 16 to 20 hours and finally put into envelopes and left to dry as in the spore-suspension method.

In the study of the spore-suspension and vacuum methods, the surface dusting method of seed inoculation also was used for comparison. This was applied by shaking seed and spores in a vial until the seed was thoroughly blackened. The inoculated seed then was removed from the vial and momentarily shaken on a fine screen to remove the excess of smut spores.

The field plantings were made in 1936 and 1937 at Moscow, and Sandpoint, Idaho, and Ithaca, New York, in 5-foot rows spaced 1 foot apart. Each year a single lot of smut was used for all of the inoculations. It consisted of a physiologic race of *Ustilago hordei* to which the varieties used in the tests were susceptible. These varieties were Hannchen (C.I. 531), Odessa (C.I. 934), and Trebi (C.I. 936).

RESULTS

In the first test, conducted in 1936, all of the seed, previous to inoculation, was treated with formaldehyde solution (1 to 320) for 1 hour. The treated seed was then immediately rinsed in running water for one-half hour and spread out in a thin layer to dry until restored to its original pre-treatment weight. In studies of physiologic races of *Ustilago hordei* such treatment of the differential seed lots is necessary or desirable to insure against possible foreign inoculum. As a result of treatment and drying, the hulls frequently split (Fig. 1) and become more or less loose around the caryopsis, due, probably to dissolution of the sticky substance noted by Harlan (6) that

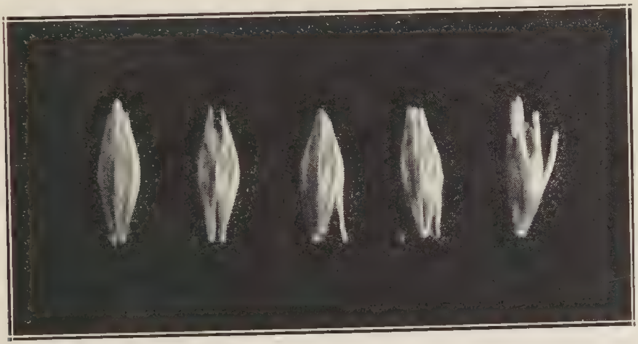

FIG. 1. Trebi barley showing (left) an untreated kernel with hulls intact and (right) four kernels with hulls loosened and split as a result of treatment with formaldehyde solution for 1 hour followed by washing in water and drying.

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oats, and noted that the suspending medium had little effect on spore germination or promycelial growth on the oats.

causes the glumes to adhere to the kernels. This facilitates the lodgment of spores beneath the seedhulls and as noted later (Table 2), markedly increases the effectiveness of inoculation. The seed was sown in duplicate sets at Ithaca, N. Y., and in quintuplicate sets at Moscow and at Sandpoint, Ida. (Table 1). As shown in the table, the average percentages of smutted heads for the 3 varieties at the 3 places were as follows: Superficial dusting method 16.8 per cent, spore-suspension method 46.9 per cent, and vacuum method 49.5 per cent.

TABLE 1.—*Comparative effectiveness of three methods of inoculating seed barley with covered smut (Ustilago hordei) at three experiment stations in 1936*

Variety	Location	Method of inoculation					
		Seed dusted with dry spores		Spore-suspension		Spore-suspension under vacuum	
		Total heads	Smutted heads	Total heads	Smutted heads	Total heads	Smutted heads
		Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Hannchen	Moscow, Ida.	1,890	14.9	1,626	39.3	1,826	36.2
"	Sandpoint, Ida.	448	23.4	633	55.9	454	50.7
"	Ithaca, N. Y.	200	9.0	177	33.3	165	41.8
Odessa	Moscow, Ida	1,346	18.9	1,375	55.8	1,440	66.2
"	Sandpoint, Ida.	422	36.3	519	70.5	333	74.8
"	Ithaca, N. Y.	160	10.6	227	39.2	231	42.9
Trebi	Moscow, Ida.	971	12.0	956	38.0	869	42.0
"	Sandpoint, Ida.	287	15.0	382	50.5	252	67.1
"	Ithaca, N. Y.	237	6.3	268	23.5	194	30.9
Total or average		5,961	16.8	6,163	46.9	5,764	49.5

In 1937, the experiment was conducted along similar but more comprehensive lines. In order to determine the rôle of the loosened seedhulls resulting from seed treatment with formaldehyde solution followed by washing in water and drying, all 3 inoculation methods were applied to non-treated as well as formaldehyde-treated seed. Also, a part of each inoculated seed lot was treated with 50 per cent copper carbonate dust immediately before the seed was sown.

The experiment was sown in triplicate at Moscow, Ida., and Ithaca, N. Y., and in duplicate at Sandpoint, Ida. The results were similar at all three stations and are combined in Table 2.

The data show that the pre-treatment of seed with formaldehyde solution, followed by thorough washing and drying before seed inoculation, resulted in almost a doubling of the average percentages of smutted heads from the 3 different methods of inoculation. It also resulted in relatively more effective control with copper carbonate dust. Apparently the loosening of hulls around the caryopsis enabled more inoculum and more copper carbonate to get beneath the hulls, thus increasing the effectiveness of inoculum in infection and of copper carbonate in control. The copper carbonate treatment also was far more effective when applied to seed inoculated by the

TABLE 2.—*Average covered smut infection in three varieties of barley inoculated and treated by different methods and sown at three experiment stations in 1937*[a]

Method of seed inoculation	Seed treatment		Hannchen		Odessa		Trebi		All varieties	
			Heads		Heads		Heads		Heads	
	Before inoculation	After inoculation	Total	Smutted	Total	Smutted	Total	Smutted	Total	Smutted
			Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Noninoculated check	None	None	[b]	Tr.[c]		0.0		Tr.		Tr.
Seed blackened with dry spores	“	“	2392	9.7	1652	15.6	1422	6.5	5466	10.6
“ “ “ “ “	“	Copper carbonate	2415	1.2	1579	2.5	1442	1.5	5436	1.7
Spore suspension	“	None	2268	20.9	1403	29.1	1378	13.8	5049	21.2
“ “	“	Copper carbonate	2357	18.2	1629	20.1	1353	16.9	5339	18.4
Spore suspension under vacuum	“	None	2451	23.4	1701	26.5	1346	21.0	5498	23.8
“ “ “ “	“	Copper carbonate	2306	20.8	1669	24.6	1466	17.1	5441	20.9
Noninoculated check	Formaldehyde solution	None	...	0.0		0.0		0.0		0.0
Seed blackened with dry spores	“ “	“	2335	14.9	1415	26.7	1332	12.6	5082	17.6
“ “ “ “ “	“ “	Copper carbonate	2473	0.4	1493	0.7	1471	0.2	5437	0.4
Spore suspension	“ “	None	2337	34.0	1760	47.5	1428	35.7	5525	38.8
“ “	“ “	Copper carbonate	2468	22.4	1591	20.4	1452	17.2	5511	20.5
Spore suspension under vacuum	“ “	None	2393	35.6	1689	45.8	1422	38.5	5504	39.5
“ “ “ “	“ “	Copper carbonate	2298	20.3	1315	20.2	1405	19.1	5018	19.9

[a] Data are totals and averages of results at Moscow and Sandpoint, Ida., and Ithaca, N. Y.

[b] Total heads in checks, not counted.

[c] Tr. = trace, approximately 0.1 per cent smut.

surface dust method than when applied to seed inoculated by the spore-suspension and vacuum methods. In 1937, as in the previous year, the spore-suspension and vacuum methods were approximately equally effective, and both were far superior to the surface dust method.

During the past 7 years the senior writer has used the spore-suspension method in studies of physiologic races of *Ustilago hordei* (13) and in further inquiry into the practicability of the method and its effectiveness under different field conditions. The experiments, conducted at Ithaca, N. Y., involved the inoculation of 2,000 or more individual lots of seed each year. Although the inoculated barley was subjected to extremes of drought, precipitation, or heat during this period, the infections each year have been adequate for the differentiation of physiologic races. In the susceptible variety, Odessa (C.I. 934), the maximum of smutted heads in the 7 years ranged from 48 to 69 per cent with an average for all years of approximately 59 per cent. The method also proved practicable. The fact that it is a wet method is a distinct advantage in that spores of the different smut collections under test are kept from flying about and becoming a source of contamination. The writers have not used the vacuum method in extensive tests for physiologic races; but, from the experience of the present study, it would appear more difficult to apply. Placing in the vacuum jar the vials containing seed covered with spore suspension requires time. Also, it involves the hazard of contaminations through spattering of the spore suspensions when the vacuum is applied. In large-scale inoculations involving many individual smut collections, the spore-suspension method, therefore, would seem more practicable.

A few additional reports on the methods used herein, or somewhat similar methods, are available. At Sandpoint, Ida., according to Hungerford (7), the vacuum and spore-suspension methods of seed inoculation, respectively, resulted in 82 and 70 per cent of covered smut. Allison (1) and Leukel (10) also used vacuum methods that produced better results than did surface dusting of seed with dry spores. Tapke (12) obtained satisfactory results with the spore-suspension method in inoculating barley with the seedling-infecting loose smut *Ustilago nigra*. In winter barleys, up to 83 per cent of smutted heads were obtained.

It is evident from the foregoing results that relatively simple and consistently effective methods of inoculating seed barley with covered smut are finally available. Studies on physiologic races of *Ustilago hordei* and breeding for resistance in barley are now going forward.

SUMMARY

Two methods of inoculating seed barley with covered smut, the spore-suspension and vacuum methods, were devised and tested under field conditions at Moscow and Sandpoint, Ida., and Ithaca, N. Y. Both proved far more effective in smut production than the well-known method of inoculating seed by coating the surface with spores.

The spore-suspension and vacuum methods involve three essential features: (1) The seed is first treated for an hour with formaldehyde solution, then washed in water, and dried. This treatment eliminates superficially borne foreign inoculum, loosens the hulls around the caryopsis, and materially increases the effectiveness of inoculation; (2) the seed is covered with spores in suspension. Spores are thus carried beneath the hulls and come to lie close to the point of attack as in the effective natural inoculation; (3) inoculated seed is stored 16 to 20 hours while moist. This promotes spore germination and spread of inoculum before the seed is dried and sown.

The vacuum method has been slightly superior to the spore-suspension method in smut production but with large scale inoculations, particularly in studies of physiologic races, the latter method appears to be easier and safer to apply.

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CONTROL OF THE COMMON MOSAIC DISEASE OF TOBACCO BY BREEDING[1]

W. D. VALLEAU

(Accepted for publication March 11, 1942)

Fifteen years ago it was found that the common mosaic of tobacco could be controlled successfully by insisting that the hands of the workers who chewed or smoked barn-cured tobacco be freed from mosaic virus before weeding and pulling plants,[2] but, because of difficulties where hired help is used, growers are not always successful. It is, therefore, highly desirable that resistant varieties of tobacco, equal in other respects to those commonly grown, be produced. With this object in mind, the writer has conducted breeding studies on Burley and dark tobacco in an attempt to produce desirable mosaic-resistant varieties. Three promising lines are being followed: (1) hybridizing desirable varieties with *Nicotiana digluta* (seed obtained from R. E. Clausen, University of California), and repeatedly backcrossing; (2) hybridizing desirable varieties with Ambalema and repeatedly backcrossing; (3) repeatedly backcrossing the best Ambalema-type resistant plants (A) on glutinosa-type resistant plants (N) to combine the two types of resistance.

Recently, objections have been raised to the use of the N factor in control of tobacco mosaic,[3] but in the experience of the writer the conclusion that "it does not seem that the glutinosa type of mosaic resistance has any practical value" seems entirely unwarranted.

Theoretically, the glutinosa (N) type of resistance, when transferred to commercial varieties of tobacco, should be ideal. It is governed by a dominant gene; consequently, backcrosses can be made on selected plants in every generation. Thus is should be a relatively simple matter, if the N gene has been transferred to a tabacum chromosome, as Holmes seems to believe,[4] to introduce resistance of this type into any number of commercial varieties of tobacco. The backcrossed strains, after about the third or fourth backcross, usually appear nearly identical with the commercial variety used as the backcross parent. It should then be necessary only to self a strain that has been repeatedly backcrossed and select plants homozygous for the N factor. The original variety plus the resistance factors should result. The writer has isolated 3 homozygous strains from a fifth backcross strain prepared by Holmes in which Ky. 16 was used. Two of the NN strains were of lighter color than Ky. 16, had smaller upper leaves, and bloomed much lower. The suckers were very tough and hard to remove. The third strain was of the same color as Ky. 16, grew nearly as large, but yielded about 25 per cent less tobacco. Several other homozygous strains of Burley of the writer's own breeding have been isolated but nearly all have been discarded because of slow growth, low yield, or other undesirable characters. These results seem to indicate that when the N factor from *Nicotiana glutinosa* is well established in the tabacum genom, it still carries with it other factors that, when in a homozygous condition, markedly affect the type of plant produced. Some NN strains appear desirable, suggesting that, eventually, satisfactory varieties may evolve.

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[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] Valleau, W. D., and E. M. Johnson. Observations and experiments on the control of true tobacco mosaic. Kentucky Agr. Expt. Stat. Bull. 280, 143–174. 1927.

[3] Clayton, E. E., and H. H. McKinney. Resistance to the common mosaic disease of tobacco. Phytopath. 31: 1140–1142. 1941.

[4] Holmes, F. O. Inheritance of resistance to tobacco-mosaic disease in tobacco. Phytopath. 28: 553–560. 1938.

As to the value of the N factor in the control of tobacco mosaic, there seems little doubt, in spite of objections that have been raised to it. There is no question that seedlings transplanted to thumb pots usually will be destroyed if heavily inoculated, making it necessary to remove the inoculated leaf soon after the necrotic spots develop, if the plant is to be saved. Rapidly growing plants in the field may likewise be destroyed if *heavily* inoculated. In the writer's field tests the past season, 27 strains of Nn or NN Burley tobacco were inoculated June 18, 1941, 25 days after setting. Inoculum used consisted of freshly crushed green leaves, applied with the thumb and fingers to the tip of one upper leaf. Of 441 N plants, 435 developed necrotic spots but otherwise remained healthy (Nn or NN); only 6 developed systemic necrosis Nn or NN). Inoculation was undoubtedly heavier than would ordinarily occur in accidental inoculation by the farmer. In addition, 3 NN strains of Burley were tested extensively with farmers in 1940 and 1 strain in 1941. These strains were not introduced for general planting, but were used for the purpose of demonstrating that mosaic can be controlled by the use of a resistant variety. In some of these demonstrations, the grower inoculated both the resistant and susceptible plants, but in no instance has a case of systemic necrosis been reported. An occasional grower, who has been much troubled by mosaic in the past, has grown a crop of *NN* tobacco with complete success as far as mosaic is concerned.

Another point should be kept in mind in connection with the use of the necrotic response in mosaic control: Nearly all mosaic infection originates on the hands of the workers, either as a result of handling viruliferous dried tobacco or, in rare cases, handling diseased weeds in the plant bed.[5] Assuming, for argument's sake, that every plant, inoculated with tobacco mosaic virus at weeding, pulling, and setting time, develops systemic necrosis and dies, experience with susceptible varieties has shown that it would be rare indeed that 10 per cent of the plants in the field would be affected following setting. Field evidence indicates that there would be no further spread from necrotic plants the remainder of the season because of low virus content in necrotic plants that may survive. The crop at harvest time would be virtually virus-free, and would not, therefore, carry virus over winter.

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[5] Valleau, W. D., and E. M. Johnson. Tobacco mosaic—sources of infection and control. Kentucky Agr. Exp. Stat. Bull. 376. 1937.

Therefore, any chewing or smoking tobacco from the crop, or any trash used for fertilizer from it, would be virus-free, and the second-year crop should be entirely uninjured by the virus. Granting that soil carry-over may sometimes be a minor factor in field infection, there should be none following an N-resistant crop because of the few plants affected and because of the very low virus content of the necrotic plants. In warm regions, where tobacco plants sometimes survive the winter and act as a source of mosaic for the succeeding crop, there is little likelihood that necrotic plants would survive. The mosaic disease in an N-resistant crop thus is self-eradicatory in contrast with its self-perpetuating habit in a susceptible crop.

With Burley tobacco it has been possible to backcross with a susceptible variety 4 consecutive times on Ambalema-resistant F_2 Burley plants and still maintain resistance. There is some question as to whether resistance is of as high a degree as occurred in the original Ambalema selections. Backcross resistant seedlings develop some mottling 2 or 3 weeks after inoculation and may become slowly and nearly completely invaded if grown to maturity; yet the growing-point leaves of rapidly growing plants are unmottled and undistorted, indicating a high degree of resistance. When plants of this degree of resistance are grown in the field and inoculated with a bleaching strain of the mosaic virus as soon as rapid growth commences, the majority remain healthy, except for local chlorotic lesions, while a part develop an occasional chlorotic ring pattern on one or more lower uninoculated leaves. In commercial plantings where mosaic is abundant in a susceptible variety, these resistant varieties remain entirely free from any noticeable infection. The objections to these resistant Burley varieties, noted so far, are that they have a somewhat objectionable plant type, even after repeated backcrossing; yield has been lower than is obtained from the Burley parent (Ky. 16) used in backcrossing; and the majority of the hybrids wilt in hot weather, with the result that one or more leaves scald. Ky. 16 seems to be completely free from this trouble. It is possible that these objections may be overcome. The quality of certain of the resistant strains appears to be satisfactory.

Theoretically, there may be some objections to the A type of resistance in that resistant plants sometimes carry a slight amount of virus, which might act as a source of inoculum for tomatoes or other succeptible crops when the viruliferous tobacco is prepared and sold in commercial form. Actually such a large percentage of A-resistant plants escape systemic infection, when heavily inoculated in the field, that there is little danger of any resistant plants developing systemic infection under farm conditions. There is a real danger, however, that strains of the mosaic virus may develop that will become systemic in N plants and produce a mottle disease, rather than necrosis, as Blood and Watson report in *Datura meteloides*.[6] Both of these theoretical difficulties can be overcome, and nearly immune strains of tobacco produced, if the N and the A types of resistance are combined in one variety. Ten such strains of Burley were set in the field in 1941 and inoculated 25 days after setting. All were heterozygous for N, and had not been selected for A-type resistance, except that A-type resistant plants were used in back-crossing. Of a total of 294 plants inoculated, 245 showed no evident signs of infection, 46 developed systemic mottle mosaic, and 3 developed a few chlorotic ring patterns on lower leaves. In tests later in the summer it was usually difficult to detect N plants because of slow development of local necrotic spots in plants in which the 4 recessive *a* factors also were present. In the greenhouse, tender *Nn aaaa* plants develop local necrotic spots but rarely manifest systemic necrosis under conditions where nearly all Nn plants would be destroyed. In a few instances *Nn aaaa* plants that developed systemic necrosis recovered completely when set in the ground bench.

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[6] Blood, H. L., and R. D. Watson. A modification of the tobacco mosaic virus No. 1 occurring in *Datura meteloides*. Utah Acad. of Sci., Arts and Letters 15: 15–19. 1938.

From the writer's experience it seems safe to conclude that both the Ambalema (A) and the glutinosa (N) types of resistance, either singly or together, will prove satisfactory for practical control of tobacco mosaic if satisfactory commercial varieties containing these factors in a homozygous condition can be produced.

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THE COMPOSITION AND FIELD PERFORMANCE OF SOME SILVER SPRAYS

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(Accepted for publication March 14, 1942)

During the first fifteen years of the present century several attempts were made to use various silver compounds and mixtures as plant sprays. Though there was no consistent success with any of these, probably the most promising was a silver nitrate-soap mixture.[3] An effort has been made recently by the senior writer[4] to develop fungicidal sprays with silver as the toxic component. These developmental studies were conducted under laboratory and greenhouse conditions. It is the purpose of this paper to present the composition and field performance of some of the more promising silver sprays developed in the laboratory.

THE SILVER SPRAYS

One of the promising mixtures was prepared by adding a silver nitrate solution to a solution of sodium lauryl sulphate.[5] This spray will be referred to as the silver-lauryl sulphate mixture. The silver nitrate concentration in this mixture varied between 0.299 and 1.195 g. per gal., while the sodium lauryl sulphate concentration (as Dreft or IN–181–P) varied from 6.3 to 7.5 g. per gal. Two other silver sprays had 3 components. They contained, in common, silver nitrate and hydrated lime. The third component in each case was either ferrous sulphate or manganous sulphate. Laboratory studies indicate that the following relative concentrations of the 3 components produce mixtures whose residues are most adherent to potato foliage: (A) the silver-ferrous sulphate mixture containing silver nitrate 0.598 g., ferrous sulphate ($FeSO_4 \cdot 7H_2O$) 0.98 g., and hydrated lime (73 per cent calcium hydroxide) 0.59 g. per gal. of spray mixture; (B) the silver-manganous sulphate mixture containing silver nitrate 0.598 g., manganous sulphate ($MnSO_4 \cdot 4H_2O$) 0.77 g., and hydrated lime (73 per cent calcium hydroxide) 0.59 g. per gal. of spray mixture.

The ferrous sulphate and manganous sulphate mixtures were each prepared by simultaneously adding the suspended hydrated lime and dissolved sulphate salt to the solution of silver nitrate. The complete addition

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[3] Vermorel, V., and E. Dantony. Le mildiou de la grappe. Prog. Agr. et Vit. 31: 101–102. 1910; Abstract in Rev. Vit. 34: 71. 1910.

[4] Nielsen, L. W. Studies with silver compounds and mixtures as fungicidal sprays. In press.

[5] Commercial sources of sodium lauryl sulphate:

Dreft Orvus W. A. Paste	Procter and Gamble Co., Cincinnati, Ohio.
IN–181 IN–181–P	E. I DuPont de Nemours & Co., Wilmington, Delaware.

of the sulphate solution to the silver nitrate may precede that of the hydrated lime without any visible material change in the spray. However, the available data[6] indicate that the simultaneous addition of the sulphate solution and the hydrated lime suspension will produce the more adherent mixture. The resulting mixture in each case is black, and has an amorphous precipitate similar to that of Bordeaux mixture.

Of the 3 sprays, the silver-ferrous sulphate mixture was the most adherent to potato foliage in the laboratory tests; silver-lauryl sulphate mixture was the least adherent. Due to an excess of sodium lauryl sulphate, the silver-lauryl sulphate mixture readily wets the sprayed foliage. However, in order to wet foliage having a waxy cuticle, wetting agents must be added to the ferrous- and manganous-sulphate mixtures. Since silver ions have an affinity for proteins, wetting agents containing proteinaceous materials should be avoided.

The field experiments were concomitant with the laboratory developmental studies. Thus, in the field tests the concentrations of components of any silver spray mixture and the silver compound itself have varied somewhat from year to year as progress was made in the laboratory. For this reason the above described spray mixtures, which recent laboratory studies indicate are best, are not included in the field experiments reported here.

THE FIELD EXPERIMENTS

During the summer of 1938, the silver-lauryl sulphate mixture, silver oxide, and a colloidal-like silver, were compared with Bordeaux mixture and with red cuprous oxide (as a spray) for the control of late blight of celery (*Septoria apii*). The copper concentration in the red cuprous oxide mixture was equivalent to that in the 4–4–50 Bordeaux mixture. The silver nitrate concentration for each of the silver sprays was 0.598 g. per gal.

TABLE 1.—*Data from a field experiment comparing three silver sprays and two copper sprays for the control of Septoria apii infection of celery*

Spray	Mean number of marketable* leaves per plant	Difference† between Bordeaux mixture and other treatments
Bordeux mixture (4–4–50)	7.19	..
Silver-lauryl sulphate	6.68	−0.51
Colloidal silver (a) (b)	5.23	−1.96**
Silver oxide (a) (c)	5.2	−1.99**
Red cuprous oxide	5.08	−2.11**
Control	2.55	−4.64

* Marketable leaves means those leaves either free of infection or having mild infection. Severely infected and dead leaves were counted but not considered in this analysis.

† Minimum difference for odds of:

* 19 : 1	1.43 leaves
** 99 : 1	1.95 leaves

(a) Lethane 1/600 was used as a spreader.
(b) Silver nitrate reduced with stannous chloride.
(c) Produced by adding silver nitrate solution to sodium hydroxide solution.

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[6] See footnote 4.

The experimental plants were artificially inoculated July 11, 1938. By July 30 good infection had developed and the first spray application was made. The plants at that time were developing their 4th or 5th leaves. Each spray treatment was applied to 27 ft. of row and was replicated 4 times. Eight applications of spray were made at weekly intervals. The data were obtained by examining carefully every 4th plant in each row to determine the number of disease-free or mildly infected leaves. Both are grouped together as marketable leaves. The data are summarized in table 1. During the time of this experiment there was a total precipitation of 8.49 inches.

Of the silver sprays the silver-lauryl sulphate mixture gave best late-blight control, and the control obtained with this spray was nearly as good as that obtained with 4–4–50 Bordeaux mixture. No visible residue was left by the silver-lauryl sulphate spray and no injury was observed.

The silver-lauryl sulphate mixture at concentrations of 0.299 and 0.598 g. of silver nitrate per gal. was compared with 5–2.5–50 Bordeaux mixture for the control of late blight of potatoes during the summer of 1939. In 1940 silver-manganous sulphate with 0.299, 0.598, and 1.195 g. of silver nitrate per gal. and silver-ferrous sulphate with 0.598 g. of silver nitrate per gal. were compared with Bordeaux mixture. In both experiments the silver sprays were significantly less efficient than 5–2.5–50 Bordeaux mixture in controlling late blight.[7]

During the season of 1940 various spray materials were compared for the control of tulip "fire," caused by *Botrytis tulipae*. In previous experiments copper-containing sprays in general proved injurious to tulips. High-lime Bordeaux mixture (1.5–4.5–50) was comparatively safe, while 4–4–50 Bordeaux mixture caused serious injury to foliage with consequent reduction in bulb growth. Of the sprays tested in 1940 only the silver-lauryl sulphate, silver-manganous sulphate, and the 1.5–4.5–50 Bordeaux mixture were statistically better than the check when compared on the basis of bulb weight increase. The other sprays will not be considered further here. The silver sprays contained 1.195 g. of silver nitrate per gal. The silver-lauryl sulphate mixture contained 6.8 g. of IN–181–P (sodium lauryl sulphate) per gal. The silver-manganous sulphate mixture contained 3.79 g. of manganous sulphate and 6.8 g. of hydrated lime per gal. Each treatment was replicated 8 times. Spray applications were made April 26, May 7, May 23, and June 2, 1940, and were applied before predicted rain periods. Prevailing conditions were extremely favorable for an epiphytotic of tulip "fire."

The 2 silver sprays, Bordeaux mixture and the check, were compared on the basis of (1) bulb weight increase, (2) number of leaf spots, and (3) number of flower spots. Bulb increase was calculated by subtracting the planting weight from the harvest weight and dividing the remainder by the planting weight. The leaf-spot data were taken by using a card having an opening of 1 sq. in. The square opening was placed successively on 6 leaves chosen at random in each row of the 5 rows per plot—making a count of 30 sq. in. for each plot. The flower data were taken by counting the total number of lesions on 18 flowers selected after a definite pattern, such as flowers No. 1, 3, 6, and 9 in the first row; No. 2, 5, and 8 in the second row; and No. 1, 3, 6, and 9 in the third row, etc., through the 5 rows in each plot. The data are summarized in table 2.

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[7] Unpublished data from experiments conducted by W. M. Epps, Research Assistant, Department of Plant Pathology, Cornell University, Ithaca, New York.

TABLE 2.—*A field comparison of two silver sprays and Bordeaux mixture for the control of tulip "fire" as determined by measuring bulb weight increase, leaf spots, and flower spots of sprayed plants*

Spray	Bulb weight increase in per cent of planting weight	Leaf spots	Flower spots
	Per cent	*Number*	*Number*
(a) Bordeaux mixture 1.5–4.5–50	100.6*	17.6	143.1
(b) Silver-manganous sulphate	101.0	52.3	161.6
Silver-lauryl sulphate	99.7	55.2	143.4
Check	77.1	222.7	307.1

* Numbers given in table are means of eight replications.
(a) Spreader, Vatsol OT† at a concentration of 1/650 based on solid.
(b) Spreader, Vatsol OT at a concentration of 1/1000 based on solid.
† Commercial source:

American Cyanamid and Chemical Corp.
30 Rockefeller Plaza
New York City

The silver sprays compared favorably with the 1.5–4.5–50 Bordeaux mixture in fungicidal action and had a further advantage in that they left no unsightly residue on the foliage and flowers.

Silver-lauryl sulphate, silver-manganous sulphate and silver-ferrous sulphate sprays were compared with Bordeaux mixture during the season of 1941 for the control of tulip "fire." Owing to the dry growing season very little infection developed in the check plots and no comparative protection data were obtained.

The silver-manganous sulphate mixture, as prepared for the tulip experiments, including the spreading agent, cost 2.7 cents per gallon. The Bordeaux mixture plus spreading agent cost 1.4 cents per gallon. However, the silver spray covered about 25 per cent more area because of its superior wetting properties. With a manganous sulphate concentration of 0.77 g. per gal. as given in the earlier formula (based on laboratory studies), the cost of this spray would have been approximately 2.0 cents per gallon.

SUMMARY

The composition and method of preparing three silver sprays are given.

In a field experiment there was no significant difference between silver-lauryl sulphate mixture and Bordeaux mixture in the control of late blight

of celery. The 3 silver sprays tested were not so efficient as Bordeaux mixture for the control of late blight of potatoes. Both the silver-lauryl sulphate mixture and silver-manganous sulphate mixture were as good as Bordeaux mixture for the control of tulip "fire" in one season's test.

None of the silver sprays left an objectionable residue on the sprayed foliage. If one may judge from the experiments with tulips, the silver sprays may be considered to give greater promise of value as fungicides for certain plants that are susceptible to copper injury.

FUSARIUM WILT OF RADISH

JAMES B. KENDRICK AND WILLIAM C. SNYDER

(Accepted for publication March 9, 1942)

A vascular fusarium disease of White Chinese Winter radish (*Raphanus sativus* var. *longipinnatus* Bailey) was observed in a seed field in San Benito County, California, in April, 1934.[1] This planting had been made sometime in the summer of 1933 and the disease was well advanced, indicating that it had been present the previous summer. Several plants had been killed by the disease; many others showed a yellowing and dwarfed unilateral growth. The disease has not been observed elsewhere in California, neither has it again occurred on this ranch, as the infested area has not subsequently been planted to radishes.

The general symptoms of the disease on the large radish seed plants resembled those of cabbage yellows caused by *Fusarium oxysporum* f. *conglutinans* (Wr.) Sny. and Hans. Diseased plants showed yellowing and dropping of the leaves, frequently confined to one side of the plant, a dark-brown, vascular discoloration, severe stunting, and in many cases, death of the plant. A Fusarium was readily isolated from the discolored vascular tissue of the diseased stems.

Young plants, growing in artificially infested soil in the greenhouse, also show symptoms characteristic of cabbage yellows. In 3 to 4 weeks after seedlings emerge from the soil, a unilateral yellowing of the lower leaves begins to show on one side of the plant. The yellowing of the leaves progresses rapidly from the lower to the upper leaves, and the leaves soon drop from the plant. The invasion of one side of the petiole and leaf by the fungus often results in a lateral and downward curling of the leaf. In the later stages of the disease on young plants, fungus invasion becomes general and all the leaves usually turn yellow and drop from the plant, leaving a bare stem, which soon dies.

Pathogenicity studies were made by planting seed directly in pots of steam-sterilized soil. After the seedlings were established, agar-plate cultures of the causal Fusarium were shredded and added to flasks of sterile water. A small amount of the mycelial and spore suspension was poured around the base of the plants and immediately washed into the soil by a rather heavy watering.

On July 7, 1934, a series of such inoculations were made in which 3 separate isolates of Fusarium from radish were used and White Chinese Winter radish, White Icicle radish (*Raphanus sativus* L.) and Early Jersey Wakefield cabbage (*Brassica oleracea* var. *capitata* L.) were exposed to each isolate. The first symptoms of the disease were observed 21 days after inoculation, and final records were taken on September 18, when most of the radish plants were dead. No evidence of the disease showed on the cabbage plants. The results are summarized in table 1.

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[1] Kendrick, James B., and William C. Snyder. A vascular fusarium disease of radish. (Abstract) Phytopath. 26: 98. 1936.

TABLE 1.—*Summarized results of greenhouse inoculation tests of radish and cabbage to the Fusarium responsible for the radish wilt*

Variety	Inoculated soil		Controls	
	Number of plants	Percentage diseased	Number of plants	Percentage diseased
White Chinese Winter radish	104	91.3	24	0.0
White Icicle radish	113	90.2	32	0.0
Early Jersey Wakefield cabbage	161	0.0	57	0.0

On September 22, 1934, 8 varieties of radish, Early Jersey Wakefield cabbage, and Jersey kale (*Brassica oleracea* var. *acephala* DC.) were planted in the same pots of soil inoculated July 7. The first symptoms of the disease were noted on the young seedlings on October 16, and final notes were recorded on December 20, 1934, when most of the plants were dead from the disease. The results are tabulated in table 2.

TABLE 2.—*Susceptibility of radish varieties, cabbage, and kale to the Fusarium wilt disease of radish in greenhouse inoculation trials*

Variety	Inoculated soil		Controls	
	Number of plants	Percentage diseased	Number of plants	Percentage diseased
White Chinese Winter radish	123	87.8	36	0.0
White Icicle radish	169	85.8	52	0.0
Long Black Spanish radish	38	71.0	7	0.0
California Mammoth White radish	62	90.3	20	0.0
Chinese Rose Winter radish	61	25.5	13	0.0
Scarlet Turnip radish	64	79.6	22	0.0
Long Scarlet radish	62	75.8	21	0.0
French Breakfast radish	62	35.3	23	0.0
Early Jersey Wakefield cabbage	217	0.0	77	0.0
Jersey kale	75	0.0	35	0.0

The above table shows that all types of radish tested are susceptible to the fusarium disease. The fact that the varieties Chinese Rose Winter and French Breakfast showed considerably less infection than the others might indicate some degree of resistance in these two varieties. Tables 1 and 2 also show that the Fusarium causing the radish disease does not attack cabbage or kale, both of which are susceptible to cabbage yellows.

Pure cultures of the radish Fusarium, prepared from single conidia, have shown the fungus to be a member of section Elegans.[2] Culturally it differs from the cabbage yellows Fusarium principally in the unreliable character of mycelial color, which, in case of the radish fungus, is usually a deep vinaceous. Morphologically the radish Fusarium falls into the species *F. oxysporum* Schl., as emended by Snyder and Hansen.[3]

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[2] See footnote 1.

[3] Snyder, W. C., and H. N. Hansen. The species concept in Fusarium. Amer. Jour. Bot. 27: 64–67. 1940.

Since no vascular Fusarium pathogenic on radish has been described in the writers' knowledge and since data given here demonstrate that a form of *F. oxysporum*, biologically distinct from the cabbage-yellows organism, *F. oxysporum* f. *conglutinans*, causes a wilt of radish, it is proposed that the radish organism be described as a new form as follows:

Fusarium oxysporum f. *raphani* n. f. A vascular parasite of *Raphanus sativus*, and variety *longipinnatus*, causing fusarium wilt of radish.

SUMMARY

A vascular fusarium wilt of White Chinese Winter radish (*Raphanus sativus* var. *longipinnatus*) characterized by symptoms similar to those of cabbage yellows caused by *Fusarium oxysporum* f. *conglutinans* occurred in a radish seed field in California in 1934. Pathogenicity studies have shown the causal fungus to be pathogenic to common radishes (*R. sativus*), as well as White Chinese Winter radish, and distinct from the cabbage yellows Fusarium. The designation *Fusarium oxysporum* f. **raphani** n. f. is proposed for the radish wilt organism.

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PHYTOPATHOLOGICAL NOTE

Chloropicrin as a Disinfectant for Plant Beds.—The rapid accumulation of parasitic fungi in the soil in greenhouse or outdoor plant beds, as a result of repeated plantings of the same crop, often halts further work with that crop until such soil is either disinfected or replaced. The use of steam for disinfection is cumbersome and frequently not feasible. Disinfection with some of the chemicals, recommended for this purpose, involves objectionable drenching of the soil; others are inflammable, or explosixe, and most of them render the soil unfit for planting for a considerable period. Soil replacement is laborious and often unsatisfactory.

Chloropicrin, apparently free from most of these objectionable features, has been found effective and satisfactory as a soil disinfectant by a number of investigators. Clayton[1] found it effective in tobacco beds against the black root-rot fungus, *Thielaviopsis basicola,* and the root-knot nematode, *Heterodera marioni.* Cooke[2] applied it to the soil at the rate of 200 lb. per acre and prevented injury to sugar cane by *Pythium aphanadermatum.* At the rate of 400 lb. per acre, Godfrey[3] found it effective as a fungicide for species of *Fusarium, Rhizoctonia, Sclerotium,* and certain other fungi. McLaughlin and Melhus[4] improved stands and increased yields of 6 field crops, presumably by controlling harmful soil fungi with an application of chloropicrin at the rate of 480 lb. per acre. Young[5] reported that from 300 to 600 lb. per acre prevented injury from *Fusarium lycopersici* and *Heterodera marioni* in tomatoes and also controlled weeds.

In the winter of 1940–41, the writer tried chloropicrin for disinfecting soil in part of a greenhouse bench and also in a number of soil flats. Its effectiveness in these tests led to its application in May, 1941, as a disinfectant in outdoor plant beds, 5 × 8 ft., in which Colby milo was to be planted. These beds, in which sorghum had been grown for 5 years, had become infested with species of *Pythium,* especially *P. arrhenomanes.*

Chloropicrin was applied May 12 at the rate of 3 cc. per sq. ft. with a special applicator that placed the material 6 in. deep. The soil was then lightly watered and immediately covered with a tarpaulin. After 4 days, the tarpaulin was removed and the soil spaded. On May 29, Colby milo was planted both in the disinfected beds and in similar adjacent beds that also had been watered, covered, and spaded, but had received no chloropicrin.

Emergence was slightly better in the treated soil than in the untreated controls, and the disinfected soil was free from weeds, while the untreated soil was soon overrun with them. Pronounced differences in growth of the milo plants were not observed until mid-summer, when the plants in the untreated beds began to show symptoms of the "milo-disease." By August

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[1] Clayton, E. E., J. G. Gaines, G. M. Stone, and K J. Shaw. Soil treatments for tobacco plant beds. (Abstract) Phytopath. 31: 8. 1941.

[2] Cooke, D. A. The relation of Pythium to growth failure on phosphate-fixing soils. Report of the Assoc. of Hawaiian Sugar Technologists 12: 169–198. 1933.

[3] Godfrey, G. H. Control of soil fungi by soil fumigation with chloropicrin. Phytopath. 26: 246–255. 1936.

[4] McLaughlin, J. Harvey, and I. E. Melhus. The response of some field crops on soils treated with chloropicrin. (Abstract) Phytopath. 32: 15. 1942.

[5] Young, P. A. Soil fumigation with chloropicrin and carbon bisulfide to control tomato root knot and wilt. Phytopath. 30: 860–865. 1940.

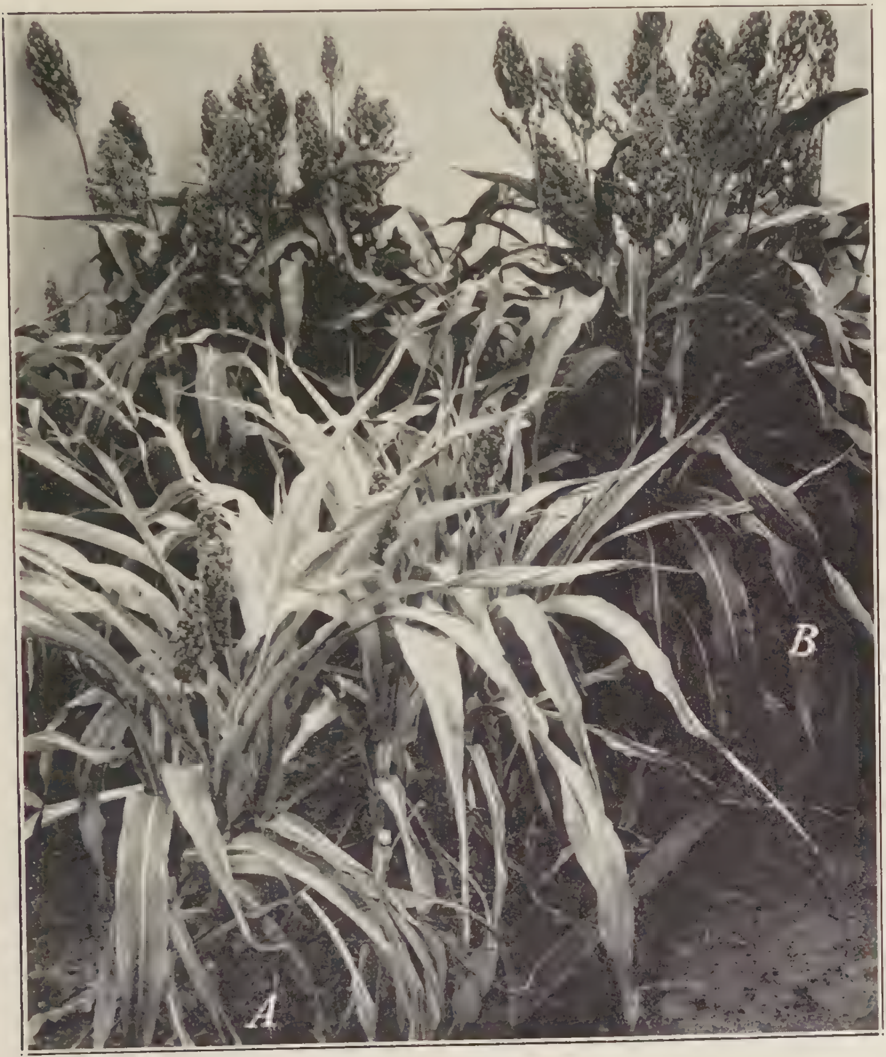

FIG. 1. Colby milo grown in *Pythium*-infested soil, which was, *A*, left untreated, and, *B*, treated with chloropicrin.

15, the contrast between the plants in the treated and untreated soils was very striking (Fig. 1). Those in the treated soil were vigorous and healthy and were producing excellent heads, whereas those in the untreated soil were stunted and dying and the heads were not filled. *Pythium arrheno-*

manes was readily isolated from the crowns and roots of these diseased plants.

Chloropicrin should not be used in greenhouses in which plants are growing, because growing plants are very sensitive to it. Complete directions for its application may be obtained from the manufacturers.—R. W. LEUKEL, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

A RING-SPOT TYPE OF VIRUS DISEASE OF TOMATO[1]

R. W. SAMSON[2] AND E. P. IMLE[3]

(Accepted for publication May 5, 1942)

A virus disease of the tomato, characterized, in the most conspicuous stages, by intricate patterns of necrotic rings and lines on young leaves and necrotic streaks on the stems of infected plants, occurs in field and garden plantings throughout Indiana. Fruits faintly to conspicuously marked with concentric, brown, corky, necrotic rings of varying size and extent sometimes occur on the diseased plants. The disease was first noted in July, 1930, at Vincennes, Indiana, and has since been observed on as high as 90 per cent of the plants in many canning-tomato fields in the southern part of the State. Scattered infected plants have been noted as early as mid-July, but the disease is most abundant on field tomatoes in August and September. Its occurrence in Missouri has been confirmed by inoculations from several collections of diseased tomatoes received through the courtesy of C. M. Tucker of the Missouri Agricultural Experiment Station. A single collection was made in Illinois in August, 1941. The virus has been secured from vaguely mottled plants of horse nettle (*Solanum carolinense* L.) growing in tomato fields, indicating that this may be a commonly occurring perennial weed host. Plants of *Nicandra physalodes* (L.) Pers. and *Datura stramonium* L. infected with the virus have been found in and near tomato fields. The disease must be somewhat destructive to the tomato crop, but the extent of damage has been difficult to estimate because the extensive epidemics so far observed have been coincident with serious defoliation caused by *Septoria lycoperisici* Spegg. and *Alternaria solani* (E. and M.) Jones and Grout. The effects of the fungous diseases have overshadowed those of the virus.

The necrotic rings produced on the fruit, foliage, and stems of tomato and on the leaves of other hosts suggest the name, tomato ring spot, for the disease. Only brief references to the writers' work on it have been published previously (3, 11).

SYMPTOMS OF RING SPOT ON TOMATO

The conspicuous feature of a tomato plant naturally affected with ring spot in the field is the curling and extensive necrosis of the terminals of one or more actively growing shoots. Brown, clearly defined, necrotic rings and sinuous lines appear on the basal portions of leaflets of the younger leaves (Fig. 1, A). The petioles of the necrotic leaves and the adjacent portions of the stems are frequently marked by necrotic streaks and rings. Under greenhouse conditions, small, brown, necrotic rings may appear within 6 to

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[1] Journal Paper No. 24, of the Purdue University Agricultural Experiment Station, Lafayette, Indiana. Contribution from the Department of Botany.

[2] Assistant Chief in Botany, Purdue University Agricultural Experiment Station.

[3] Formerly Graduate Assistant in Botany, Purdue University Agricultural Experiment Station.

12 days on inoculated leaves of young tomato plants, but such local lesions do not always result. Three to 6 days later the typical ring-and-line patterns appear on the young leaves and stem tips, sometimes in sufficient intensity to kill the terminals. The length of the incubation period is less at 70° and 80° than at 62° F. Resumption of growth of the terminals or of one or more lateral buds of affected shoots usually takes place promptly after the development of the necrotic phase of the disease, both under greenhouse and outdoor conditions. Such new growth shows either no symptoms or only a vague mottling, and appears to develop and fruit normally.

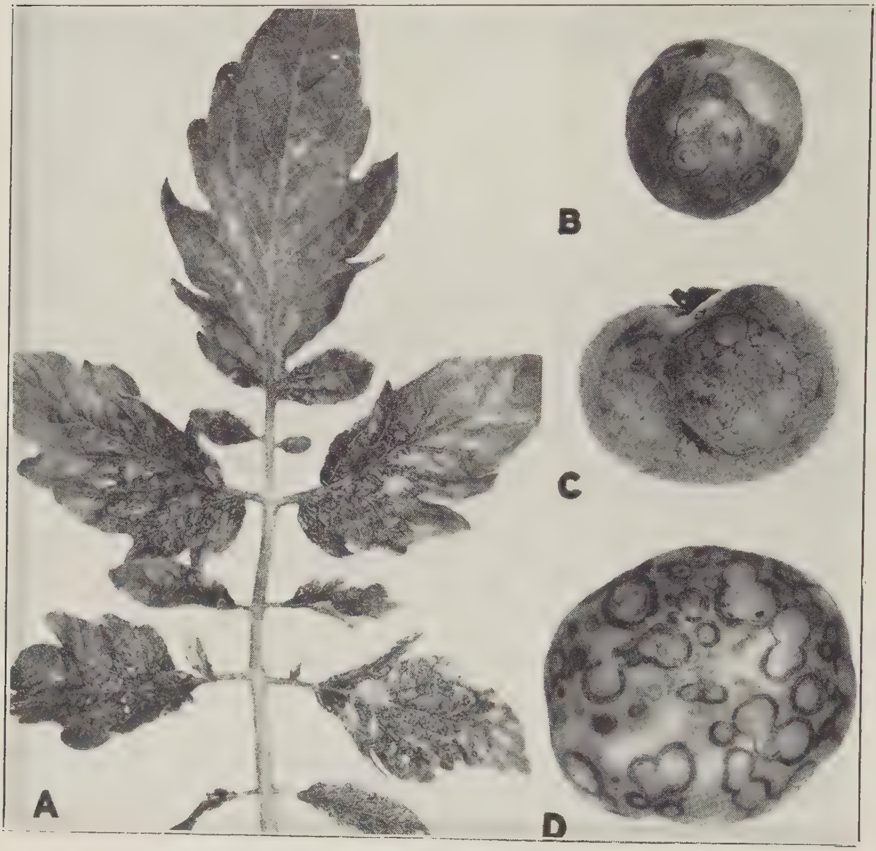

FIG. 1. Tomato ring spot on leaf and fruits of tomato. A. Network of brown, necrotic lines on basal portions of leaflets. B–D. Faint to conspicuous grey or brown, corky, superficial, concentric rings or portions of rings on green fruits.

The symptoms that sometimes occur on fruits in the field vary from faint to conspicuous grey or brown, corky, superficial, frequently concentric rings or portions of rings (Fig. 1, B–D). They apparently develop only on those fruits that are very young at the time of virus invasion. They are suggestive of the fruit symptoms described by Samuel, Bald, and Pittman (12) and Parris (8) for the Australian spotted wilt disease, and Milbrath (7) for the tip blight disease, but differ in being superficial and corky in nature. The presence of the ring-spot virus has been demonstrated repeatedly in ring-marked fruits and symptomless fruits from diseased plants. Symptoms have not yet been secured on fruits of plants artificially inoculated with ring-spot virus, either in the greenhouse or field, despite numerous attempts.

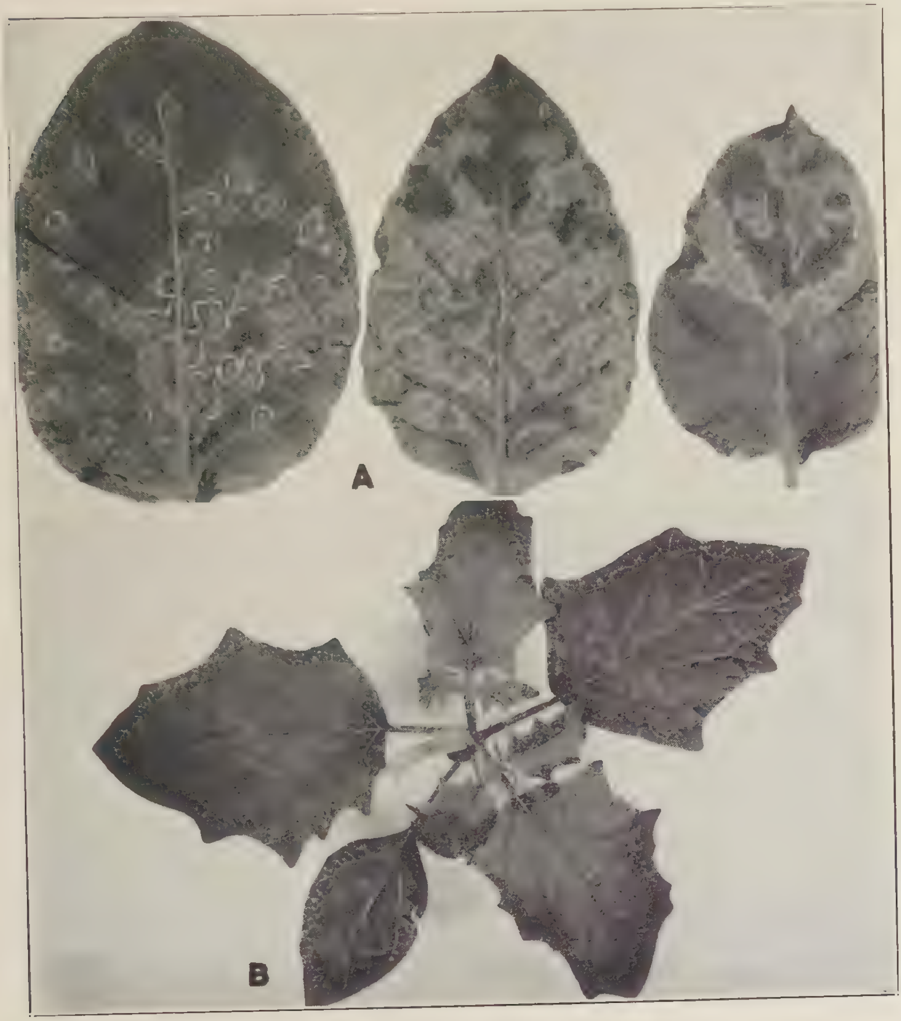

FIG. 2. Tomato ring spot on Turkish tobacco (*Nicotiana tabacum* L.) and jimson weed (*Datura stramonium* L.). A. Conspicuous ring-and-line patterns on tobacco leaves. B. Clearing and slight necrosis of veins, flecking, and inconspicuous ring-and-line patterns on leaves of jimson weed.

SYMPTOMS OF TOMATO RING SPOT ON TOBACCO AND JIMSON WEED

Young plants of Turkish tobacco (*Nicotiana tabacum* L.), infected with tomato ring-spot virus, exhibit a mild ring-and-line pattern on the inoculated leaves and somewhat more conspicuous patterns on one or more leaves next above (Fig. 2, A). Subsequent growth on such plants may show no such patterns but only a slightly paler color and a somewhat more rigid appearance than normal. Presence of the virus in both the basal and terminal leaves of such plants has been demonstrated by return inoculations to tomato and jimson weed.

Symptoms of tomato ring spot on *Nicotiana affinis* T. Moore consist of numerous, faint, small rings on the lower leaves only, although the virus may be recovered by inoculations from the terminal leaves of such plants. No symptoms of local or systemic infection have appeared on plants of *N. glutinosa* L. or *N. sylvestris* Speg. and Comes inoculated with tomato ring spot.

Indefinite rings and lines appear on the inoculated leaves of young jimson weed plants (*Datura stramonium*), followed by a clearing of the veins, flecking, and rather inconspicuous ring-and-line patterns on the next younger leaf tissues (Fig. 2, B). Subsequent leaf growth is almost devoid of any symptoms of disease, although definitely invaded by the virus.

TRANSMISSION OF TOMATO RING SPOT

Some difficulty was encountered in transmitting tomato ring spot to various hosts by leaf rubbing, unless carborundum powder was applied to the leaves prior to inoculation. The virus is easily thus transmitted to jimson weed. Several hundred attempts, all unsuccessful, were made to transmit the earlier collections of ring spot in plant extract from this host back to tomato. They were, however, transmitted with ease to tobacco, thence back to tomato. They also could be transmitted directly from jimson weed to tomato by grafting. Later, several collections were obtained that could be transmitted directly from jimson weed to tomato in plant extract without intermediate transfer through tobacco.

FAILURE TO TRANSMIT RING SPOT WITH THRIPS

Attempts were made to transmit tomato ring spot with thrips (apparently, *Thrips tabaci* Lind.). Thrips were confined for periods of 10 days to 9 weeks on virus-infected tomato and jimson weed. The insects were then transferred to feeding traps on the leaves of healthy plants of tomato and jimson weed and fed for varying intervals. In some instances they were transferred successively to 4 to 6 new plants after several hours of feeding on each plant in the series. None developed ring spot. The 9-week period should have been long enough for a new generation of thrips to have developed and become viruliferous (6, 10).

Parallel attempts to transmit a suspected culture of the spotted-wilt virus with the same stock of thrips used in the ring-spot inoculations were likewise unsuccessful.

HIGH TEMPERATURES FAVOR DEVELOPMENT OF TOMATO RING SPOT

Relatively high greenhouse temperatures favor the development of ring spot on tomato. Two different experiments were performed wherein lots of 20 plants were inoculated and placed in each of 3 greenhouse sections maintained at approximately 62°, 70°, and 80° F., respectively. The disease appeared at about the same time on the plants at the two higher temperatures and much more quickly than at 62° F. It was most severe on the plants in the 80° section, killing 75 per cent of the plants in one of the experiments.

Infected tomato plants, grown in a glass-enclosed compartment held at a constant temperature of 88° F., reacted differently, however; becoming stunted and marked with an unusual yellow mosaic pattern. Under these conditions infected jimson weed developed only a few pale rings on the inoculated leaves but no further symptoms. After one month in this compartment the plants were removed to a greenhouse section held at 68° F. Necrotic ring spots developed on young leaves and shoots of the tomato plants after 30 days, while the jimson weeds showed no further evidence of disease. The virus was subsequently recovered from the younger tissues of both species.

HOST RANGE OF TOMATO RING SPOT

Tomato ring spot has been found in the field on the varieties Greater Baltimore, Century, Clark's Early, Kalb, King Humbert, Marglobe, Pride of Illinois, Pritchard, and Rutgers. The varieties Comet, Early Detroit, Earliana, Gulf State Market, Oxheart, and South Australian Dwarf Red were successfully inoculated with the virus.

Seventy-eight species, representing 27 plant families, were tested for susceptibility to tomato ring spot. Five plants were inoculated from 2 or 3 tomato plant sources and from one jimson weed source, making a total of 15 or 20 inoculated plants of each species or variety. Return inoculations were made to tomato and jimson weed to determine systemic infection, regardless of whether the test plants showed symptoms of disease or not. Symptoms were secured on the following 2 species of the Amaranthaceae, one of the Martyniaceae, and 19 species or varieties of the Solanaceae:

Family	*Genus and Species*	*Common Name*
Amaranthaceae	*Amaranthus tricolor* L.	
	A. retroflexus L.	Pigweed
Martyniaceae	*Martynia louisiana* Mill.	Martynia
Solanaceae	*Androcera rostrata* (Dunal) Rydb.	Buffalo burr
	Capsicum frutescens L.	Pepper, Ruby King
	Datura stramonium L.	White flowered jimson weed
	D. tatula L.	Purple flowered jimson weed
	Datura sp. (horticultural form)	Vaughan's variety, Cornucopia
	Hyoscyamus niger L.	Henbane
	Lycopersicon esculentum Mill.	Tomato, 10 varieties
	L. pimpinellifolium (Jusl.) Mill.	Red currant tomato
	Nicandra physalodes (L.) Pers.	Apple of Peru
	Nicotiana tabacum L., var. Turkish	Tobacco

Genus and Species	*Common Name*
N. affinis T. Moore	
Petunia hybrida grandiflora Vilm.	Giant Ruffled Petunia
P. hybrida Vilm.	Howard's Star Improved Petunia
P. axillaris BSP.	Petunia
Physalis alkekengi L.	Chinese lantern plant
Solanum melongena L.	Eggplant
S. pseudo-capsicum L.	Jerusalem cherry
S. tuberosum L.	Potato
S. carolinense L.	Horse nettle

The ring-spot virus was recovered from all of the above by return inoculation to jimson weed and tomato, except from *Amaranthus retroflexus, Martynia louisiana, Capsicum frutescens, Hyoscyamus niger,* and *Solanum pseudo-capsicum.*

The following species failed to develop symptoms or to yield the ring-spot virus on return inoculation to jimson weed and tomato:

Family	*Genus and Species*	*Common Name*
Aizoaceae	*Tetragonia expansa* Murr.	New Zealand spinach
Apocynaceae	*Vinca rosea* L., 2 varieties	Periwinkle
Asclepiadaceae	*Asclepias tuberosa* L.	Milkweed
Begoniaceae	*Begonia semperflorens* Link & Otto	Begonia
Chenopodiaceae	*Chenopodium album* L.	Lamb's quarter
	Beta vulgaris L., 2 varieties	Garden beet
	B. vulgaris var. *cicla* L.	Swiss chard
Compositae	*Ambrosia artemisiifolia* G.	Ragweed
	Zinnia elegans Jacq., 2 varieties	Zinnia
	Bidens bipinnata L.	Spanish needle
	B. discoidea (T. & G.) Britton	Spanish needle
	Emilia sonchifolia DC.	Tassel flower
	Helianthus annuus L.	Sunflower
	Erigeron speciosus DC.	Erigeron
	Calendula officinalis L., 2 varieties	Calendula
	Callistephus chinensis Nees.	China aster
	Tagetes signata Bartl.	Marigold
	T. erecta L.	Marigold
Convolvulaceae	*Ipomoea batatas* Lam.	Sweet potato
	I. purpurea Lam.	Morning glory
	Quamoclit penata Bojer, 3 varieties	Cypress vine
Cruciferae	*Brassica oleracea* L., 2 varieties	Cabbage
	B. alba Rabenh., 2 varieties	Mustard
	B. rapa L., 2 varieties	Turnip
Cucurbitaceae	*Lagenaria leucantha* Rusby	Dipper gourd
	Luffa cylindrica Roem.	Dish cloth gourd
	Cucurbita pepo L., 3 varieties	Nest egg gourd, Golden Summer Crookneck squash, Cocozelle Bush squash
	C. moschata Duchesne	Cushaw pumpkin
	C. maxima Duchesne	Hubbard squash
	Cucumis melo L., 2 varieties	Cantaloupe
	C. sativus L., 2 varieties	Cucumber
	C. anguria L.	West Indian gherkin
	Citrullis vulgaris Schrad., 2 varieties	Watermelon
Dipsaceae	*Scabiosa atropurpurea* L.	Scabiosa
Euphorbiaceae	*Euphorbia heterophylla* L.	Mexican fire plant

Family	*Genus and Species*	*Common Name*
Geraniaceae	*Pelargonium hortorum* Bailey	Pelargonium
	P. odoratissimum Ait.	Pelargonium
Leguminosae	*Lupinus hartwegii* Lindl.	Lupine
	Phaseolus vulgaris L.	Golden Cluster wax bean
	P. limensis Macf.	Lima bean
	Glycine max Merr., 2 varieties	Soybean
Malvaceae	*Abutilon theophrasti* Medic.	Indian mallow
	Hibiscus esculentus L.	Okra
Phytolaccaceae	*Phytolacca decandra* L.	Pokeweed
Plantaginaceae	*Plantago lanceolata* L.	Bracted plantain
	P. major L.	Common plantain
Polemoniaceae	*Phlox drummondii* Hook., 2 varieties	Phlox
Polygonaceae	*Rumex acetosella* L.	Red sorrel
	Pesicaria hydropiperoides Michx.	Smartweed
Ranunculaceae	*Delphinium ajacis* L., 2 varieties	Larkspur
Scrophulariaceae	*Antirrhinum majus* L., 2 varieties	Snapdragon
	Verbascum thaspus L.	Mullein
Solanaceae	*Lycopersicon peruvianum* (L.) Mill.	
	Nicotiana glutinosa L.	
	N. sylvestris Speg. & Comes	
	Salpiglossus sinuata L., 2 varieties	Salpiglossus
Tropaeolaceae	*Tropaeolum majus* L., 2 varieties	Nasturtium
Umbelliferae	*Daucus carota* L.	Carrot
Violaceae	*Viola tricolor* L., 2 varieties	Pansy

PROPERTIES OF THE TOMATO RING-SPOT VIRUS

Tolerance to Heat. Two milliliter portions of infectious tomato juice, filtered through cheesecloth, were placed in small, thin-walled test tubes and suspended for 10-minute periods in a water bath at various temperatures; they were then immediately inoculated into young tomato plants. Ring-spot infection was secured from preparations heated at 56° but not at 58° C. or higher.

Resistance to Aging in Vitro. Quantities of juice from ring-spot-infected tomato plants held in test tubes at laboratory temperatures were infectious after 21 hours, but not after 27 hours, when inoculated into jimson weed. Tomato plants were infected from such juice stored for 21 hours at 3° C. but only jimson weed plants were infected with juice stored for 45 hours at this temperature. Complete inactivation occurred after 60 hours storage at 3° C.

Limits of Dilution. The tomato ring-spot virus in tomato juice was found to be infectious only at relatively low dilutions. Preparations diluted to 1 : 250 were infectious while those diluted to 1 : 500 were not.

Desiccation of the Ring-spot Virus in Infected Plant Tissue. Ring-spot infection was secured from water extracts of tomato leaves dried in the light in a warm greenhouse for 114 hours but not for 300 or 530 hours. The virus was destroyed more quickly by drying in tobacco leaves than in tomato leaves.

COMPARISON OF TOMATO RING SPOT WITH SPOTTED WILT, TOBACCO RING SPOT, POTATO RING SPOT, AND RING MOSAIC

Spotted Wilt. Differences in symptoms produced, properties, and host range distinguish the tomato ring-spot virus from the spotted-wilt virus. The writers have compared three different collections of spotted wilt with tomato ring spot in the greenhouse. The symptoms produced on tomato by these collections have been in almost complete agreement with those described by Samuel, Bald, and Pittman (12).

Bronzing is not a characteristic symptom of tomato ring spot on tomato. The necrotic symptoms of ring spot on tomato fruits (Fig. 1, B–D) seem to be more clearly defined, when they do occur, than those described by Samuel, *et al.*, (12) and Parris (8) for spotted wilt.

The tomato ring-spot virus survives a temperature of 56° C. for 10 minutes, as compared to inactivation of the spotted wilt virus at 42° C. (1). The tomato ring-spot virus survives aging *in vitro* for 21 hours at room temperatures, while the spotted wilt virus is reported to lose its infectiousness after 6 hours at such temperatures (1). This, however, may not be a sufficient difference in tolerance to aging to distinguish the two viruses.

The host range of tomato ring spot does not appear to be as extensive as that of spotted wilt. *Calendula officinalis, Zinnia elegans, Scabiosa* sp., *Lupinus* sp., *Phaseolus vulgaris, Plantago major, Delphinium* sp., *Antirrhinum majus, Nicotiana glutinosa, N. sylvestris, Salpiglossus* sp. and *Tropaeolum majus* are listed as hosts of the spotted-wilt virus (13). None of these were successfully infected with the ring-spot virus.

Parris (8) and Sakimura (10) present evidence that a virus causing a ring spot of tomato is the same as that of pineapple yellow spot and spotted wilt. The host range, symptoms produced, thermal death point and transmission by *Thrips tabaci* indicate the tip-blight virus, described by Milbrath (7), to be very similar to, if not identical with, the spotted-wilt virus and distinct from tomato ring spot.

One cross-protection test was made, in which 8 rooted and rapidly growing cuttings from tomato plants infected with the tomato ring-spot virus were inoculated with a suspected culture of the spotted-wilt virus. Typical symptoms of spotted wilt appeared on all cuttings 7 days after inoculation. That all of the cuttings were infected with tomato ring spot, even though none showed any symptoms, was demonstrated by inoculating young seedlings from them prior to inoculation with spotted wilt. The spotted-wilt virus was secured from a diseased tomato plant received from L. J. Alexander of the Ohio Agricultural Experiment Station, who collected it in an Ohio greenhouse. Attempts to transmit it with the onion thrips failed, but otherwise it agreed with published descriptions of spotted wilt (8, 12, 13). It was transmitted to tomato, jimson weed, Turkish tobacco, *Nicotiana glutinosa, Emilia sonchifolia*, aster, zinnia, snapdragon and nasturtium, with the development of typical symptoms. In parallel inoculations only the first three of the above were successfully infected with the tomato ring-spot virus used to inoculate the tomato plants from which the cuttings were taken.

Tobacco Ring Spot. Differences in transmissibility, symptoms produced and host range serve to distinguish the tomato ring-spot virus from the tobacco ring-spot virus (9, 14). The ring-and-line patterns usually produced by it on Turkish tobacco are broader and more severe in their effects than those produced by the tomato ring-spot virus. Tobacco ring spot has been transmitted to such species as *Tetragonia expansa, Beta vulgaris, Chenopodium album, Ambrosia artemisifolia, Bidens discoidea, Calendula officinalis, Helianthus annuus, Tagetes erecta, Zinnia elegans, Ipomoea purpurea, Citrullis vulgaris,* various species of *Cucumis, Scabiosa atropurpurea, Phaseolus vulgaris, Phytolacca decandra, Nicotiana glutinosa,* and *Antirrhinum majus,* (14), none of which could be infected with the tomato ring-spot virus.

Price (9) compared the host ranges of 6 plant viruses, one of which he refers to as tomato ring spot. He reports *Tetragonia expansa, Vinca rosea, Beta vulgaris, Helianthus annuus, Zinnia elegans, Cucumis sativus, Pelargonium hortorum, Phaseolus vulgaris, Phytolacca decandra, Phlox drummondii, Nicotiana glutinosa* and *N. sylvestris* as susceptible to this virus. No symptoms were produced on any of these hosts by the writer's tomato ring-spot virus, nor could it be recovered from any of them. Symptoms produced by a culture of Price's tomato ring spot on tomato, jimson weed and Turkish tobacco were strikingly different from those produced by the writer's tomato ring spot.

Potato Ring Spot. The tomato ring-spot virus commonly occurs in tomato plants in the field showing symptoms of tobacco-mosaic infection. This suggested that the disease might be due to the combined effects of the two viruses, or that the tomato ring-spot virus might be the same as the potato ring-spot virus (5), which acts in complementary fashion with the tobacco-mosaic virus to produce the necrotic disease, tomato streak (2, 15). Since the tobacco-mosaic virus does not become systemic in *Datura stramonium,* it has been possible to inoculate this plant from tomato plants showing symptoms of both ring spot and tobacco mosaic, and subsequently transfer the ring-spot virus, freed of tobacco mosaic, back to tomato. Typical ring-spot symptoms have been secured repeatedly on tomato plants so inoculated. Likewise, the disease has been obtained, free of tobacco mosaic, on several occasions by direct inoculation from naturally infected tomato and *Solanum carolinense* plants.

Tomato plants infected with, and showing symptoms of potato ring spot are not protected against subsequent infection by tomato ring spot. Typical symptoms of the latter have been secured on such tomato plants.

Ring Mosaic. Johnson (4) described a ring mosaic of tobacco that produces concentric, sunken, chlorotic rings on tomato fruits and streaking of the stems. The fruit symptoms are not characteristic of tomato ring spot. The other symptoms produced by the ring-mosaic virus on tomato, the resistance of the virus to drying, and the fact that it does not become systemic in jimson weed further distinguish it from the tomato ring-spot virus.

SUMMARY

A virus disease of the tomato, characterized by intricate patterns of necrotic rings and sinuous lines on young leaves, streaks on the stems, and faint to conspicuous, concentric, brown, necrotic rings of varying size and extent on the fruit, occurs in field and garden plantings throughout Indiana. The symptoms produced on tomato and other hosts suggest the name, tomato ring spot, for the disease.

Infected tomato plants outgrow the necrotic phase of the disease and subsequently show either no detectable symptoms or only a vague mottling. The virus is present in the symptomless parts of such plants.

Attempts to transmit tomato ring spot with *Thrips tabaci* Lind. (onion thrips) were unsuccessful and inconclusive.

Under greenhouse conditions the disease develops more rapidly and is more destructive at a temperature of 80° than at 70° or 62° F.

Seventy-eight species, representing 27 plant families, were tested for susceptibility to tomato ring spot. Disease symptoms were produced on two species of the *Amaranthaceae*, one of the *Martyniaceae*, and on 19 species and varieties of the *Solanaceae*. It was recovered, by return inoculation to jimson weed and tomato, from *Amaranthus tricolor* and from 16 species and varieties of the *Solanaceae*.

Studies of the properties of the virus in tomato-plant extract indicate that: (a) its thermal death point is between 56° and 58° C., (b) it survives aging *in vitro* for 21 hours but not 27 hours at room temperatures, and for 45 but not 60 hours at 3° C., (c) it is infectious at dilutions of 1:250 but not 1:500, and (d) it survives desiccation in detached tomato leaves exposed in a warm greenhouse for 114 but not 300 hours.

Differences in symptoms of disease, host ranges, and properties clearly indicate that the tomato ring-spot virus is distinct from the tobacco ring-spot. potato ring-spot, and ring-mosaic viruses. Symptoms produced by it on tomato are highly suggestive of those produced by the spotted-wilt virus on this host, but it has a more restricted host range and a higher thermal death point. Tomato plants affected with tomato ring spot were not protected from infection by a suspected culture of the spotted-wilt virus.

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RELATIVE RESISTANCE OF ALFALFA SPECIES AND VARIETIES TO RUST CAUSED BY UROMYCES STRIATUS[1]

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INTRODUCTION

Alfalfa rust (*Uromyces striatus* Schroet.) is widely distributed in the United States, but usually has been considered one of the minor diseases. In recent years, however, it has wrought considerable damage, especially to the seed crop, in parts of central United States. The only practical means of control seems to be the breeding of resistant varieties through selection and hybridization. The studies herein reported were undertaken to obtain information on methods of culturing alfalfa rust in the laboratory, greenhouse, and nursery and to test varieties, selections, and species of alfalfa in search of sources of resistance.

This disease is of little economic importance in some regions, but in other areas it is regarded as one of the most important leaf and stem diseases. During the last 25 years, 1916 to 1940, inclusive, alfalfa rust was reported 29 times, either in the supplements or in the regular numbers of the *Plant Disease Reporter* (1, 5, 6, 7). Workers report varying degrees of damage, depending mainly on the region in question and the prevailing environmental conditions. Damage in any one season depends largely upon the amount of rainfall during the summer months and the later part of the growing season. In nearly all cases alfalfa rust seems to do its greatest damage to the crop that is being grown for seed. Under favorable conditions, however, it may be conspicuous in the later hay cuttings in the western Mississippi valley. From all indications the disease produces greater losses in the warm, humid parts of the United States than in the cooler areas.

Few reports were found in the literature on resistance to alfalfa rust. During the summer of 1911, plants in the Iowa Experiment Station plots were examined (4). One plot of an unnamed selection with nearly white flowers was found severely rusted, but an adjoining plot of a recent introduction from Germany was sparsely infected. In 1922 several plants in a severely infected field of alfalfa growing on the Soils and Crop Experiment Farm at Lafayette, Indiana, showed high resistance (3). These were transplanted to the greenhouse, where their rust resistance was proved by inoculation. Mains (2) stated in 1926 that plants resistant to alfalfa rust had been discovered in strains of such varieties as Grimm, Cossack, Vale, Argentine, Dakota, and New Zealand.

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EXPERIMENTAL RESULTS

In a study of varietal resistance in the laboratory, excised alfalfa leaves lived 3 to 4 weeks in moist Petri dishes. Inoculating leaflets by applying fresh urediospores with a small camel-hair brush gave excellent infection (Fig. 1). Rust pustules appeared on leaves inoculated in this manner 2 to 3 days earlier than on potted plants placed in a moist chamber in the greenhouse and inoculated with a spore suspension. This method of inoculation proved sufficiently accurate to determine varietal and species differences in susceptibility.

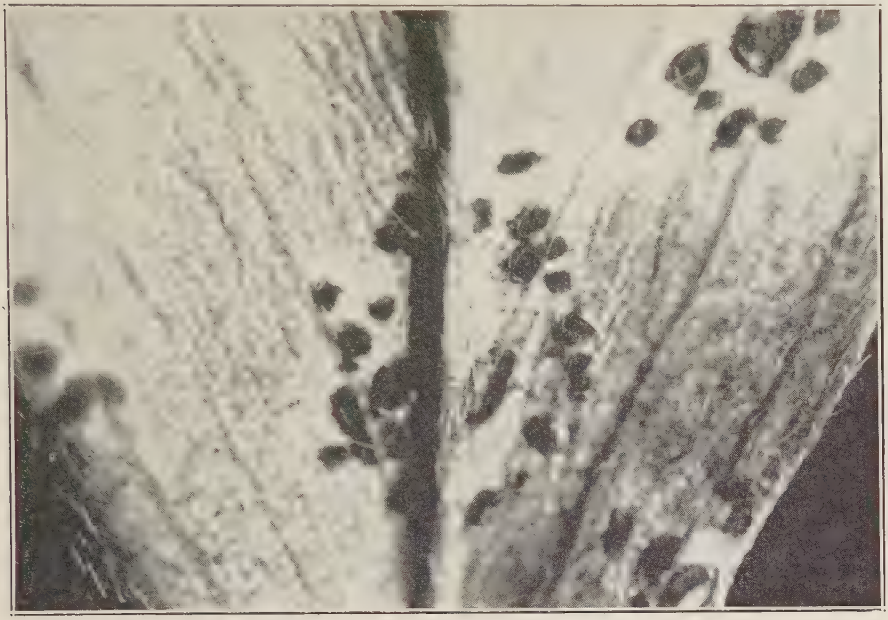

FIG. 1. Normal uredia of *Uromyces striatus* produced by the inoculation of excised leaves of Turkestan alfalfa in the laboratory.

Several methods of inoculation and incubation were tried in an effort to produce infections in the greenhouse comparable to those obtained in the field. Most methods either were too tedious or gave poor results, but after numerous trials a simple method was discovered, which, if conducted in the proper manner, yielded better results than field tests. It was found that fresh urediospores must be used to obtain satisfactory infection. A composite inoculum was prepared by scraping urediospores from infected leaves with a flattened needle. This inoculum was suspended in distilled water in the glass container of a small fly sprayer and atomized on potted alfalfa plants in moist chambers. The relative humidity was retained at close to 100 per cent for 48 hours; then the plants were removed and placed on wet sand beds on the floor of the greenhouse. Rust pustules appeared 7 to 10 days after inoculation, and final rust readings generally were taken about 3 weeks later.

TABLE 1.—*Description of infection types used in recording reaction of alfalfa varieties and species to Uromyces striatus in the greenhouse*

Host reaction	Numerical equivalent	Coefficient of severity	Type of infection	Length of infection period
Immune	0	No macroscopic evidence of infection		
Highly resistant	1	Uredia none or very few, 1 to 3 per leaflet; small, fleck-like.	Uredia, when present, non-erumpent and always in necrotic areas. Occasionally necrotic areas produced without development of uredia. Pathogen dies.	No macroscopic evidence of infection until 2 weeks after inoculation; no apparent damage to plant at 4-week period.
Resistant	2	Uredia small, scattered, 3 to 10 per leaflet.	Uredia slightly erumpent, generally surrounded by or within a necrotic or very chlorotic area.	Uredia appearing 10 to 14 days after inoculation. Host recovering from attack by end of first month.
Susceptible	3	Uredia medium-sized, 10 to 25 per leaflet. Some stem and petiole lesions.	Uredia erumpent, often surrounded by chlorotic areas, necrotic regions absent. Leaf curling and drying not evident until late, after secondary infection rings appear.	Uredia appearing 8 to 10 days after inoculation. Host and parasite develop somewhat symbiotically for a time; by end of first month leaves beginning to curl and turn brown.
Highly susceptible	4	Uredia large, abundant (25 per leaflet), often nearly covering leaf surface. Numerous stem and petiole lesions.	Uredia large and very erumpent, no necrosis or chlorosis surrounding uredia. Leaves curl and dry up rather readily. Many secondary infection rings, if leaf survives.	Yellowish, chlorotic areas appearing 4 to 5 days after inoculation, uredia evident by end of 7 to 8 days. Rust progresses rapidly and kills leaf early.

After the method of inoculation had been perfected, a varietal-testing program and search for rust-resistant types were begun. From early February to the first week in June, 1940, 2109 rust readings were made in the greenhouse tests. Plants of *Medicago ruthenica* Trantv., *M. falcata* L. and 3 different variegated alfalfas were used in this study, as well as several varieties, introductions, or selections of common alfalfa (*M. sativa*).

Some method of taking accurate notes on infected plants was necessary before a comparison of the susceptibility of varieties could be made. A 0–4 scale was adopted in order to place the rust readings on a numerical basis. The readings were made on 3 general criteria, *i.e.*, coefficient of severity, type of infection, and length of infection period. Each plant examined was given a reading under all 3 criteria, each being based upon the same 0–4 scale. The coefficient of severity denoted the degree to which the plants were infected. Type of infection corresponded to the response of the plants to rust infection, *i.e.*, the general effect of the disease on plants as manifested by chlorosis, "erumpence," etc. The length of infection period concerned mainly the time element in relation to infection. Table 1 shows the infection types and further explains the method used in recording readings on susceptibility.

The total coefficient of infection was determined by finding the sum of the figures for the 3 criteria of rust infection. Dividing the total coefficient by the total number of readings gave a figure that denoted the average coefficient of infection, or the so-called rust reading of the plant. The average of all the readings taken for the plants of a given species, variety, or selection gave the rust reading for that particular species, variety or selection. This method is believed to give an accurate measure of plant and varietal susceptibility.

TABLE 2.—*Summary showing relative susceptibility of species, varieties, and selections of alfalfa to Uromyces striatus under greenhouse conditions, Manhattan, Kans., 1940*

Species, variety or selection	Severity rank	Total plants	Total readings	Coefficient of infection	
				Total	Average
Medicago ruthenica	1	10	30	0.0	0.00
Semipalatinsk	2	24	72	58.5	0.81
Ladak	3	63	189	307.0	1.63
Medicago falcata	4	25	75	128.5	1.71
Kansas Common	5	25	75	181.5	2.42
Kansas Common 1–2018	6	74	222	547.5	2.48
Kansas Common 1–205	7	44	132	343.0	2.60
Grimm	8	77	231	210.0	2.75
Hardistan	9	10	30	97.5	3.25
Turkestan 86696	10	158	474	1552.5	3.28
Turkestan 19304	11	168	504	1681.0	3.34
Hairy Peruvian	12	25	75	267.0	3.57

A summary of the data obtained in the greenhouse (Table 2) shows the comparative resistance to rust of 12 varieties and species of alfalfa. Thus, the greenhouse tests showed the 16-chromosome species, *Medicago ruthenica,* with a 0.00 reading, to be the most resistant of all species and varieties tested. A strain of Semipalatinsk, *Medicago falcata,* which has crossed naturally with some common alfalfas since its importation from Russia, was given a rust reading of 0.81 and showed a very high degree of resistance in nearly all plants. Ladak proved to be the most resistant of any of the agronomically important varieties. *Medicago falcata* reacted variably. The results of all rust tests indicated that Kansas Common, as a variety, had only a moderate degree of rust resistance (Fig. 2), being less resistant than Ladak but considerably more resistant than Turkestan. Grimm ranked eighth among the species, varieties, and selections tested in the greenhouse, rating just below the Kansas Common strains. Turkestan types, including Hardistan, proved uniformly susceptible to rust (Fig. 3). Turkestan 86696 ranked next to Hardistan in the greenhouse tests, with a rust reading of 3.28; it was closely followed by another selection, Turkestan 19304, which had an average coefficient of infection of 3.34. Hairy Peruvian was the most susceptible variety tested in the greenhouse. Rust seemed to make its appearance on the plants earlier than on any of the other species or varieties and also caused more injury.

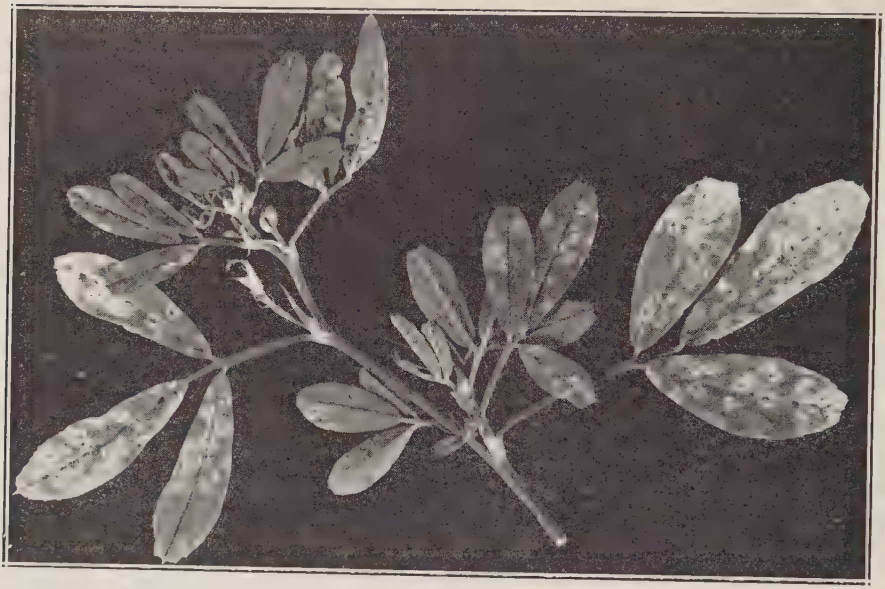

FIG. 2. Small uredia of *Uromyces striatus* on the upper surface of greenhouse-grown plants of Kansas Common alfalfa showing chlorotic areas surrounding uredia denoting moderate resistance.

The wide range in susceptibility among species and varieties of alfalfa was impressive, extending from a reading of 0.00 for *Medicago ruthenica* to 3.57 for Hairy Peruvian. The results with *M. ruthenica* might indicate that plants with a low chromosome count carry a much higher degree of resistance than those with higher chromosome numbers. Further breeding and testing are needed to clarify this point.

Notes on the severity of infection were taken in the field at different dates on 6 varieties or selections of cultivated alfalfa placed in a randomized 2-row series replicated 3 times. Plants were examined carefully for rust appearing on leaves, petioles, and stems (Fig. 4). Each row then was given a numerical rust value from 0–9, following the system of numbering used by the United States Department of Agriculture in reporting damage by the leaf-spot diseases of alfalfa. A summary of the field reactions of these varieties to rust is presented in table 3. Additional field notes were made on various varieties and selections at the Agronomy Farm plots.

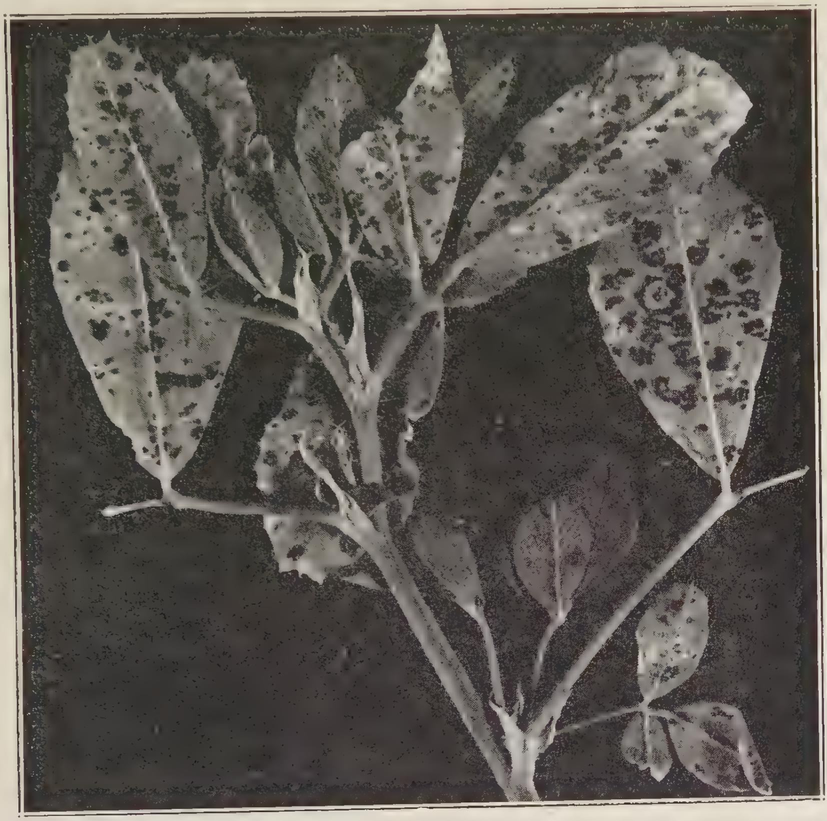

FIG. 3. Large uredia of *Uromyces striatus* on the lower surface of leaflets of susceptible Turkestan alfalfa grown in the greenhouse.

With the exception of the variety, Hairy Peruvian, field and laboratory data seemed, in general, to agree with readings made on the species, varieties, and selections in the greenhouse. Table 4 was prepared with the object of bringing all readings together, so that results could be compared directly. Here, the rankings of the various strains under each of the 3 conditions are shown. The last column in table 4 represents the final rank of each strain

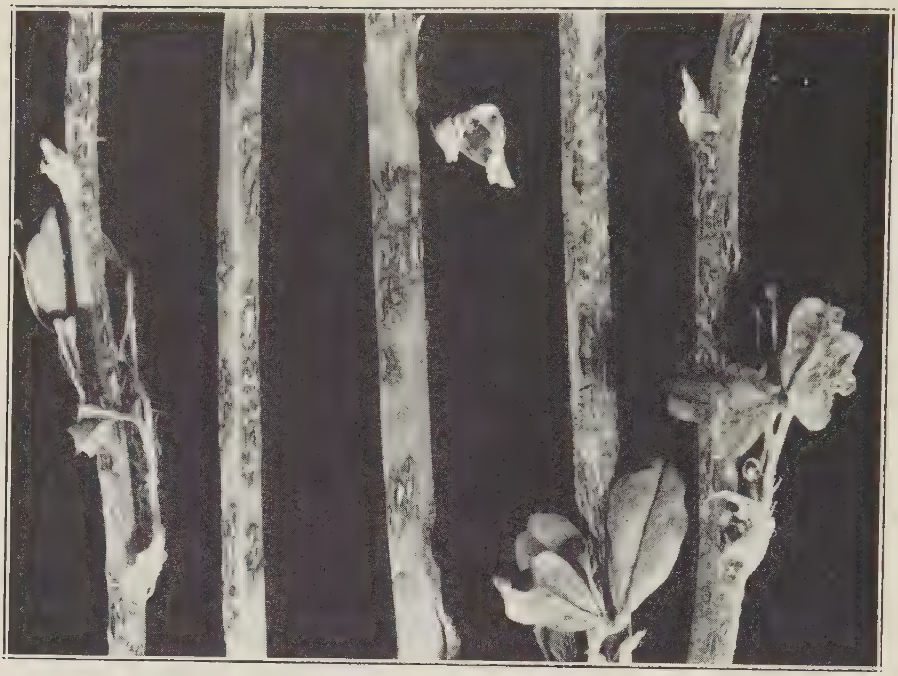

FIG. 4. Large uredia of *Uromyces striatus* on the stems of susceptible Turkestan alfalfa grown in the field.

and indicates its relative susceptibility. Table 4, therefore, shows the rank of each species, variety, or selection for each type of test and its average severity rank for the 3 tests. On the basis of the data shown in this table, *Medicago ruthenica* was the most resistant of all plants tested. Next in rank were *M. falcata* and Semipalatinsk, both of which showed considerable resistance. Ladak ranked fourth, but it might be classed as first among the commercial varieties. Kansas Common types showed only moderate resistance in all cases, hence they all had intermediate ranks. Although Hairy Peruvian proved to be the most susceptible in greenhouse tests, the final rank was somewhat above Grimm and Turkestan. This was due to its tendency to be fairly free from rust in the field during the summer of 1939. Hardistan and Turkestan were by far the most susceptible of any plants tested under each of the three conditions.

TABLE 3.—*Summary of field reactions of alfalfa varieties and selections to rust, Manhattan, Kansas, September–October, 1939*

Variety	Total rows[a]	Total readings	Total severity	Average severity	Severity rank
Grimm	10	3	54.0	3.6	4
Ladak	10	3	39.5	2.6	1
Kansas Common 1–205	10	3	46.0	3.1	3
Kansas Common 1–2018	10	3	43.5	2.9	2
Turkestan 86696	10	3	93.5	6.2	5
Turkestan 19304	10	3	96.5	6.4	6

[a] Rows replicated, readings based on observations on two rows.

TABLE 4.—*Summary table showing relative susceptibility of alfalfa species, varieties, and selections to rust as determined by experiments conducted in the laboratory, greenhouse, and field. Manhattan, Kans., 1939–1940*

Species variety, or selection	Severity rank in greenhouse	Severity rank in field		Severity rank in laboratory		Total tests	Rank in each test on basis of twelve alfalfas tested					Total	Average rank	Final severity rank
							Green house	Field		Laboratory				
		Test I	Test II	Test I	Test II			I	II	I	II			
Medicago ruthenica	1		1		1–2	3	1.0		1.09		1.8	3.89	1.30	1
Semipalatinsk	2		5		3	3	2.0		5.45		3.6	11.05	3.68	3
Ladak	3	1	6	1	5	5	3.0	2.0	6.54	2.0	6.0	19.54	3.91	4
Medicago falcata	4		2–3		1–2	3	4.0		2.73		1.8	8.53	2.84	2
Kansas Common	5		7		4	3	5.0		7.63		4.8	17.43	5.81	6
Kansas Common 1–2018	6	2	2–3			3	6.0	4.0	2.73			12.73	4.24	5
Kansas Common 1–205	7	3		3–4		3	7.0	6.0		7.0		20.00	6.67	7
Grimm	8	4	8	3–4	8	5	8.0	8.0	8.72	7.0	9.6	41.32	8.26	10
Hardistan	9					1	9.0					9.00	9.00	11
Turkestan 86696	10	5	11	5–6	9	5	10.0	10.0	11.99	11.0	10.8	53.79	10.76	12
Turkestan 19304	11	6	10	5–6	10	5	11.0	12.0	10.90	11.0	12.0	56.90	11.38	13
Hairy Peruvian	12		4		6	3	12.0		4.36		7.2	23.56	7.85	9
Kansas 308			9	2	7	3			9.81	4.0	8.4	22.21	7.40	8

The differences in susceptibility in the field afforded an opportunity to select a promising plant of Ladak alfalfa in the plots during the fall of 1939. It was grown in the greenhouse where it was subjected to the most severe rust tests. All efforts to produce rust on the plant failed, hence, it obviously carries a high form of resistance. Cuttings were made from the parent stock, and these also proved resistant. Tests of the cuttings with blackstem, *Ascochyta imperfecta* Peck, made in cooperation with the Department of Agronomy, proved that this rust-resistant selection also is highly resistant to the blackstem disease. Therefore, it seems probable that this selection carries strong resistance to several leaf and stem diseases of alfalfa and should be valuable to alfalfa breeders desiring to use resistant parental stock in crosses. If the resistance carried by this selection could be combined with the good agronomic qualities of Turkestan, a superior strain of alfalfa might be developed for the western Mississippi valley.

SUMMARY

A general study of the relative resistance of different Medicago species and varieties and selections of *Medicago sativa* L. to alfalfa rust (*Uromyces striatus* Schroet.) was made under laboratory, field, and greenhouse conditions.

A method of inoculating excised alfalfa leaves in Petri dishes was developed in the laboratory, which was found to be sufficiently accurate to determine varietal and species differences in susceptibility.

In greenhouse experiments a simple technique was developed by which rust infection comparable to that found in the field was obtained. Fresh rust inoculum was sprayed on potted plants in a moist chamber. Rust pustules appeared 7 to 10 days after inoculation, and final rust readings generally were taken about 3 weeks later.

Marked differences in resistance to rust among varieties and species of Medicago were observed in the greenhouse. *Medicago ruthenica* carried the highest form of resistance. Turkestan and Hairy Peruvian were the most susceptible. With the exception of Hairy Peruvian, readings on species, varieties, and selections made in the greenhouse and laboratory agreed very closely with those taken in the field. This one variety appeared susceptible in the greenhouse but was relatively free from rust in the field during the summer of 1939.

A plant selection of Ladak made in the field exhibited the most resistance of any strains or varieties of the *Medicago sativa* group. This selection not only showed high resistance to rust in greenhouse tests but also recently exhibited high resistance to blackstem disease of alfalfa caused by *Ascochyta imperfecta*. Cuttings of this selection have been released to the alfalfa improvement project of the Kansas Agricultural Experiment Station, Manhattan, Kansas, for other tests and possible increase.

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QUANTITATIVE MEASUREMENT OF A STRAIN OF TOBACCO–ETCH VIRUS

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More than a dozen phytopathogenic viruses can now be measured by noting the incidence of conspicuous primary lesions on inoculated leaves of appropriate host plants. These include the viruses of tobacco mosaic (7), potato mottle (17), tobacco ringspot (13), tomato spotted wilt (18), cucumber mosaic (14), alfalfa mosaic (21), tobacco necrosis (20), turnip mosaic (6), tomato ringspot (15), tobacco streak (12), tobacco Bergerac ringspot (19), tomato bushy stunt (2), potato yellow dwarf (3), pea streak (4), and pea wilt (11).

The primary-lesion method for measurement of virus activity is considerably more efficient than the most widely applicable alternative technique, which depends on the percentages of inoculated plants that become diseased at specific dilutions of inocula. In the primary-lesion method, each inoculated leaf is capable of disclosing a number of separate infections or their absence, and thus serves in the same capacity as a set of plants in the alternative method. The relative facility with which hitherto obscure properties of viruses can be investigated when the primary-lesion method becomes applicable has led the writer to hope that many, if not all, mechanically inoculable viruses eventually might be susceptible to study by this technique if further information on their potential host ranges should become available. The results from experiments with tobacco-etch virus tend to support this view.

Tobacco-etch virus (*Marmor erodens* H.) was selected for further study, several years ago, because it was a conveniently available representative of viruses then known to be readily transmitted by mechanical means from one host to another, but not known to be capable of inducing formation of conspicuous primary lesions in any susceptible species. A search was begun for a host in which it would produce conspicuous primary lesions. The severe-etch strain (var. *severum* H.) was used, because it seemed likely to induce more severe and conspicuous manifestations of disease than the type strain (var. *vulgare* H.).

The severe strain of tobacco-etch virus was originally studied and described by Johnson (10). It induces in Turkish tobacco and other varieties of *Nicotiana tabacum* L. a disease that is characterized both by chlorotic mottling and by intricate patterns of fine white lines that appear as though etched on the surface of affected leaves; in most other known hosts it induces chlorotic mottling without etched patterns.

Primary lesions are rarely discernible in hosts infected by the severe-etch strain. They are, however, sometimes to be seen in inoculated leaves of the most commonly studied host, tobacco. There they appear as faintly chlorotic spots, as fine, white, etched rings, or as combinations of the two. These primary lesions have been made clearly visible by Bawden and Kassanis (1) through staining infected tobacco leaves with iodine; the lesions, in which there is abnormal retention of starch, then can be counted accurately. In living leaves, they are not conspicuous enough to be counted readily in this or any other host that was known before the present investigation was begun.

In the search for a useful test plant for quantitative estimation of tobacco-etch virus, more than 250 species of plants were inoculated. About one-fourth of all the tested species permitted increase of the virus in their tissues; a majority of these also showed disease manifestations. Among the species that were found susceptible, a few, but only a few, showed necrotic primary lesions as a result of infection. Of these, *Physalis peruviana* L., the Cape gooseberry, proved best for estimating virus activity, as has already been reported in an abstract (9).

The characteristics of primary and secondary lesions in *Physalis peruviana*, the influence on them of age and condition of host, season, manner of inoculation, and source of virus, as well as results of some measurements made by use of the primary lesions, form the subject of the present paper.

PRIMARY AND SECONDARY LESIONS IN PHYSALIS PERUVIANA

Primary Lesions. Inoculation of *Physalis peruviana* with the severe-etch strain of tobacco-etch virus was followed in about 5 days, under appropriate conditions, by collapse of tissues in little spots on the inoculated leaves. The spots, which constituted necrotic primary lesions, later became larger and displayed a dark brown zone of pigmentation surrounding a tan or light brown center; outside these two necrotic zones there was often a third, less well defined zone of yellowing. The lesions became increasingly conspicuous until about the tenth day after inoculation, when final counts for quantitative studies usually were made. Typical necrotic primary lesions in a partly yellowed, inoculated leaf of *P. peruviana*, 10 days after inoculation, are represented in figure 1.

Old yellowing leaves sometimes showed primary lesions as chlorophyll-retention patterns without accompanying necrosis. On other occasions they showed them as spots of chlorophyll retention with but small central areas of necrosis. In either case, if lesions were numerous, these old leaves often dropped before younger leaves were ready for final counting. Because of this, records were kept from the 6th or 7th day after inoculation until about the 10th day.

Even relatively young leaves that developed many lesions tended to become yellow and to absciss earlier than comparable healthy leaves or leaves that showed fewer lesions. This abscission of inoculated leaves, though in a way comparable to the loss of inoculated leaves of the Tabasco pepper and its derivatives when inoculated with tobacco-mosaic virus (8), differed in that it did not occur promptly enough to prevent systemic spread of virus.

Secondary Lesions. Systemic spread of the virus often induced the appearance of secondary lesions in young developing leaves about the 10th day after inoculation, sometimes earlier. The secondary lesions, when they were so few as to develop without interfering with each other, resembled the primary lesions in general appearance. In young leaves that were heavily invaded, abscission sometimes occurred too early to permit development of recognizable, discrete, necrotic lesions; wilting, however, sometimes occurred in these young leaves just before they fell from their stems.

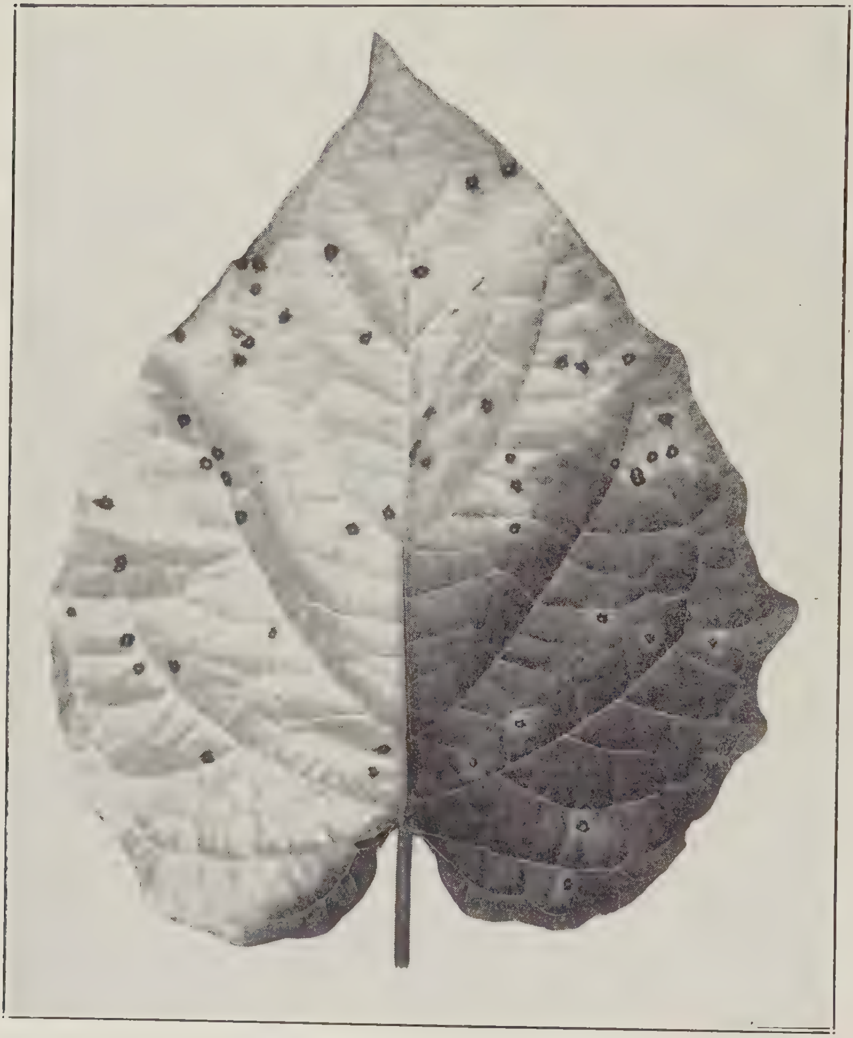

FIG. 1. Primary lesions in an inoculated leaf of Cape gooseberry, *Physalis peruviana*, 10 days after inoculation with the severe-etch strain of tobacco-etch virus. Lesions necrotic, dark brown peripherally, with lighter brown centers; in the lower right portion of the leaf, which had not yet turned yellow at the time of photographing, the green lamina was set off from each lesion by a diffuse yellow halo.

Factors Affecting the Use of Primary Lesions in Measurement of Virus

Concentrations. The type of primary lesion represented in figure 1 is perhaps the most desirable for quantitative measurement studies. It presents considerable necrosis, little or no confluence, conspicuous yellow peripheral halos in green areas of leaf tissue, high contrast in color in yellowed areas. Deviations from this type of lesion were found to occur in consequence of a number of variables that must be recognized in practical work.

Age of Test Plant. Plants proved best for use in virus estimation about the time of first flowering. Younger plants were affected so promptly by systemic necrosis as to be killed outright in a more or less diagnostic manner. They showed, in quick succession, abscission of leaves just below the top of the plant, abscission of top leaves and of the older or of all inoculated leaves, death of stem tips, and eventual collapse of the whole plant. Often the whole succession of events was so rapid that it was concluded without formation of discrete primary lesions.

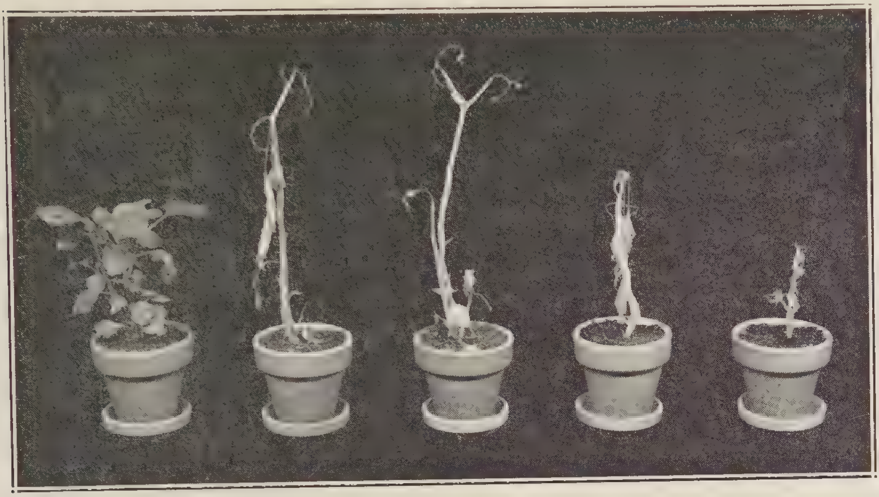

FIG. 2. Specificity of protection against the severe-etch strain of tobacco-etch virus in plants of *Physalis peruviana* inoculated with (left to right) the mild-etch strain of the same virus, healthy tobacco juice, cucumber-mosaic virus, tobacco-mosaic virus, and the Canada-streak strain of potato aucuba-mosaic virus. Only the plant at the extreme left escaped systemic necrosis as a result of the test inoculation.

Age of Inoculated Leaf. In general, plants proved less susceptible in their younger than in most of their older leaves; on some plants the very old leaves were also relatively insusceptible.

Seasonal Effects. Fall, winter, and spring conditions in the greenhouse, with temperatures held as close as possible, at least at night, to 22° C., proved satisfactory for making quantitative measurements. Summer conditions were unsatisfactory; the trouble appeared to involve unknown factors other than temperature, probably acting before inoculation. Leaves of *Physalis peruviana* grown under summer conditions were thicker and tougher than those grown in fall, winter, or spring and permitted only a few, highly localized lesions to form as a result of inoculation; natural immunity seemed to be approximated under these conditions.

Effect of Changing Strain of Virus. Conspicuous, necrotic, primary lesions like those characteristic of the severe-etch strain did not occur as a result of inoculations made with the mild-etch strain, the only other strain of tobacco-etch virus that was tested. The mild-etch strain generally induced a systemic, chlorotic-mottling disease in *Physalis peruviana.* Only occasionally some necrosis, local or systemic, was observed. Definite relationship of the two strains was shown, however, by the fact that chlorotic areas of the plants infected by the mild-etch strain showed no evidence of being infected after reinoculation with the severe-etch strain. The second inoculation produced neither necrotic primary lesions nor systemic necrosis. Similar protection was not conferred by preliminary infection with other viruses that caused chlorotic mottling in the same host, such as cucumber-mosaic virus (*Marmor cucumeris* H.), tobacco-mosaic virus (*M. tabaci* H.), or Canada-streak virus (*M. aucuba* H. var. *canadense* Black and Price). Figure 2 shows the specificity of protective inoculation with mild-etch virus. The findings confirmed the observation of Bawden and Kassanis (1) that protection against severe-etch virus is afforded by prior infection with mild-etch virus.

Effects of Intentional Variations in Manner of Making Test Inoculations. Several modifications of technique were tested to discover the best manner of conducting inoculation experiments. As a standard practice, plants of *Physalis peruviana* growing in 6-inch clay pots were inoculated, about the time of blossoming or first fruiting, by rubbing the upper surface of each large leaf with a muslin pad moistened liberally with the infectious fluid to be tested. Usually, 10 or more leaves of each plant were inoculated, and the 6 successive leaves that proved most highly susceptible were selected for counting.

Increasing the number of strokes for the inoculation of each leaf gave a slight increase followed by a decrease in primary lesions; thus with 6, 12, 18, and 24 strokes per leaf, the lesion counts per set of 12 test leaves (6 on each of 2 plants) were 1633, 1714, 2250 and 1642, respectively. Perhaps 12 strokes per leaf should be recommended as conserving time and producing numbers of lesions not likely to be greatly modified by slight variations in length or severity of strokes.

Rinsing leaves with water immediately after inoculation, 15 minutes later, or not at all, did not greatly modify results, giving averages of 38.8, 50.9, and 53.3 lesions per leaf, respectively, in a test that involved 36 inoculated leaves in each of the 3 categories. Less favorable environmental conditions or chemical constituents of inocula other than those thus far tested may, nevertheless, justify rinsing plants after inoculation.

Removing the growing tips of test plants did not appreciably affect the gradient in numbers of lesions on successive leaves; it tended to induce early appearance of secondary lesions and was discontinued.

Reducing the supply of fluid on inoculating pads, or continuing the use of pads without remoistening, resulted in unexpectedly early and progressive exhaustion of infectivity. Liberal renewal of inoculum proved essential for obtaining uniform results from successive inoculations. A freshly moistened or newly remoistened pad of cloth was generally used for inoculating each test plant, and in some experiments the inoculating pad was remoistened after inoculating each pair of successive leaves with even better results than were obtained when it was remoistened for each plant.

USE OF PHYSALIS PERUVIANA IN STUDYING SOME PROPERTIES OF THE VIRUS

Various Species as Sources of Virus. By the use of *Physalis peruviana* to measure virus activity, production of the virus by 5 common host plants was compared quantitatively, with a view to choosing a satisfactory source of virus for future experiments. Virus production in inoculated leaves 10 days after inoculation proved greatest in *Nicotiana glutinosa* L., successively less in *N. tabacum*, *Lycopersicon esculentum* Mill., *Datura stramonium* L., and *Capsicum frutescens* L. The degree to which these plants differed as virus sources in this comparison of their unmodified expressed juices may be judged from the respective counts of 1683, 698, 422, 6, and 5 lesions per set of 6 test leaves. Juices from *D. stramonium* proved more infectious when diluted 1 : 10 with water than when not diluted; therefore, the apparently low virus content of this host may be the result of a toxic effect of its juices on the test plant rather than an effect of slow or limited production of virus within its tissues. It was in this species, *D. stramonium,* that a strain of tobacco-etch virus was first recognized in nature, by Fernow (5) who called it the virus of mosaic C.

Many experiments were performed before the relative value of various species for virus production had been established. *Nicotiana tabacum,* which proved to be second in virus productivity, was used as source of virus for all of these experiments. Some later studies requiring high titer were made with material from *N. glutinosa,* however.

Rate of Increase in Tobacco. Measurement of virus from inoculated tobacco leaves and from the tops of corresponding plants at various intervals showed that inoculated leaves were, on the whole, better sources of virus than systemically infected top leaves. Activity reached a maximum in samples from both locations about 10 days after the plants' initial inoculation and then fell off, more rapidly in the systemically diseased tops than in inoculated leaves. The early increase of activity and the subsequent decline are shown graphically in figure 3. The phenomenon is comparable to that found by Ross (16) in the study of alfalfa-mosaic virus (*Marmor medicaginis* H.).

Effect of Drying. Inoculated leaves of insusceptible species of plants proved consistently free of virus when tested after 10 days, even though undiluted expressed juices from diseased plants had been applied to them originally. This led to the supposition that the virus might not survive long when dried. To test this point, juices were expressed from top leaves of plants of *Nicotiana glutinosa* 10 days after inoculation of lower leaves of the same plants. These juices were at once exposed to the air of the laboratory in 1 cc. amounts in shallow glass dishes. They became dry within an hour. Tests of infectivity were made at intervals of 0, 1, 2, 3, 5, 7, and 10 days by resuspending the air-dried residues in the original volume of water and using them as inocula for plants of *Physalis peruviana*. The results showed that complete inactivation was by no means attained within 10 days, lesion counts from 12-leaf inoculations made at the specified times being 4563, 1656, 736, 578, 519, 304, and 121, respectively. A control test of freshly expressed juice made on the 10th day gave a corresponding lesion count of 2137, lower than the count indicating original infectivity but higher than any subsequent measurement in the series. The lower value of the control perhaps reflects in part the difficulty of duplicating a sample of infective juice at or near maximum infectivity, and in part a gradual decrease in susceptibility of a set of test plants with age. The experiment shows that infectivity of expressed juice is gradually decreased by drying, but that a substantial infectivity still remains after a lapse of at least 10 days, a period in which the

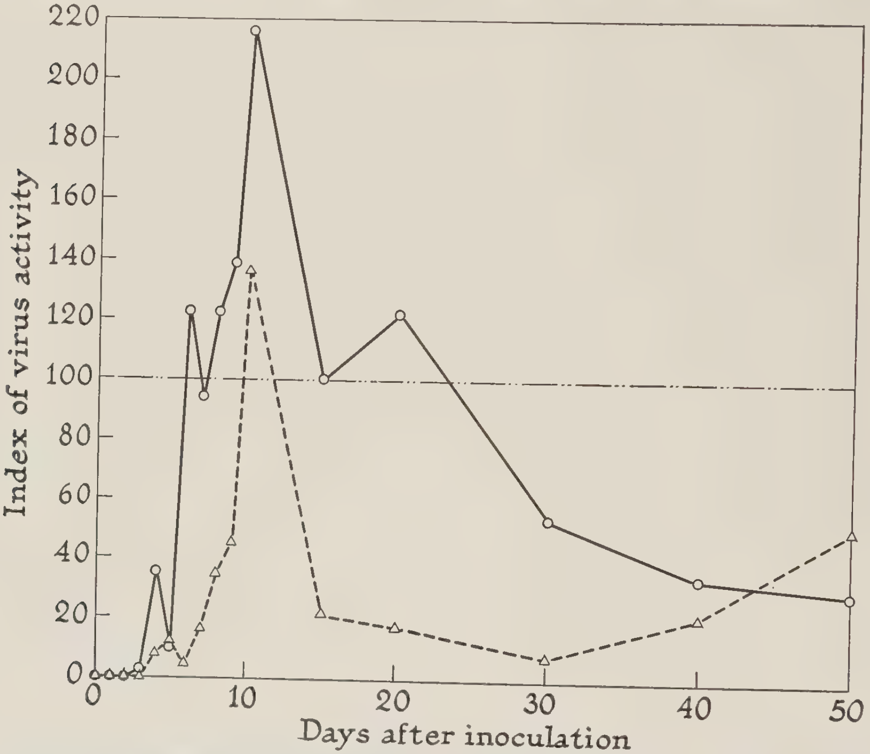

FIG. 3. Infectivity of juices expressed from inoculated leaves (solid line) and top leaves (dotted line) of tobacco plants at intervals after inoculation with the severe-etch strain of tobacco-etch virus. The index that is represented expresses a quotient: the number of lesions on a plant of *Physalis peruviana* inoculated with the indicated sample divided by the number of lesions on a control plant inoculated on each occasion with juice from a tobacco leaf infected 10 days earlier.

virus might, under some circumstances, be transported intentionally or unintentionally over considerable distances.

It became of interest to test whether virus would survive equally well within dried tissues of diseased leaves. Similar source leaves were dried, therefore, without being crushed. The drying process required more time than had been needed for small juice samples. Leaves were leathery in texture and brittle in parts after 1 day of drying in the air of the laboratory, brittle throughout after 2 days, but continued to lose weight even after the 3rd day; by the 5th day they had reached a weight that was subsequently maintained. The final air-dry weight amounted to 10.0 per cent of the original green weight. Tests of infectivity were made by remoistening and grinding the drying leaves at the intervals used in the dried-juice experiment. Lesion counts from 12-leaf inoculations in this series were somewhat lower: 3174, 122, 3, 0, 38, 31, and 9, respectively, with a corresponding control count of 397 from a fresh-leaf sample tested on the 10th day of the experiment. Again it appeared that considerable reduction of activity was experienced during a 10-day period of drying, but that dried diseased leaves remained capable of furnishing some virus for this length of time at least.

Effect of Storage at Low Temperatures. Juice samples, diluted 1:10 and stored at low temperatures in the presence of acid buffer solutions (pH range between 4.5 and 6), retained infectivity well. A sample giving an original 6-leaf lesion count of 72 still was capable, after 10 days just above freezing temperature in a refrigerator, of giving a count of 43, and, after a like period just below freezing temperature in a cold-storage room, of giving a count of 51. Both in the chilled and in the frozen sample sedimentation occurred, leaving a clear supernatant fluid of very low virus content; almost all the surviving virus seemed to be associated with the sedimented fraction.

TABLE 1.—*Inactivation of severe-etch virus by heat. Numbers indicate lesions produced on single plants of Physalis peruviana inoculated with samples of undiluted juices from severe-etch tobacco plants, after the samples had been heated at various temperatures and for the indicated intervals of time*

Heating period	51° C.	53° C.	55° C.	57° C.	59° C.
0 min.	237	436	402	311	417
5 "	31	14	2	0	0
10 "	30	6	0	0	0
15 "	27	2	0	0	0
30 "	18	0	0	0	0

Effect of Dilution. Upon water dilution of expressed juice from diseased plants of *Nicotiana glutinosa*, infectivity for *Physalis peruviana* was progressively decreased. Dilutions proved weakly infective at 1:1000, uninfective at 1:10,000. Successive dilution of 1:1, 1:2, 1:10, 1:100, 1:1000, and 1:10,000 gave lesion counts of 277, 98, 47, 7, 0.7, and 0 per set of 6 test leaves. Had a more concentrated original sample been used, the apparent dilution end-point might have been somewhat different from that found in this experiment.

Thermal Inactivation of Virus. Undiluted juice from severe-etch tobacco plants remained infective after exposure for 10 minutes at a temperature of 53° C., but was completely inactivated by exposure for the same length of time at 55° C. When the time of heat treatment was extended to 30 minutes, some infectivity could be demonstrated at 51° C., though not at 53° C. nor at any higher temperature that was tested. These and other details of the results of heat treatments are shown in table 1.

SUMMARY

Conspicuous necrotic primary lesions were produced in leaves of *Physalis peruviana,* under some environmental conditions, 5 to 10 days after inoculation with the severe-etch strain of tobacco-etch virus. Quantitative measurements of this virus could be made by using the number of lesions resulting from each inoculation as an index of the infectivity of the inoculum.

By the use of this method of measuring the virus, several plant hosts were compared as potential virus sources; *Nicotiana glutinosa* appeared to be the best among them. *N. tabacum* and *Lycopersicon esculentum* furnished less infective extracts but were fairly satisfactory sources 10 days after initial inoculation. Virus activity in expressed juices of *N. tabacum* reached a maximum about 10 days after inoculation and then declined rapidly.

Severe-etch virus was found capable of withstanding drying for at least 10 days in juice samples and in diseased leaves, although in both cases there was a reduction of infectivity with increased time. It retained activity well for at least 10 days in acid-buffer solutions (pH 4.5 to 6) at temperatures just above and just below freezing. It retained some infectivity after being heated for 10 minutes at 53° C., but was completely inactivated in this length of time at 55° C.; it was still viable to some extent after 30 minutes at 51° C., but was wholly inactivated when exposed to a temperature of 53° C. for the same period.

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BLUEBERRY CANE CANKER

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A canker disease of stems and shoots of blueberries (*Vaccinium* spp.) was first observed[2] in a North Carolina cultivated field in 1938. Later surveys showed the disease widely distributed in cultivated fields of the highbush swamp blueberry (*V. australe* Small) in North Carolina and Georgia and in cultivated rabbit-eye blueberry (*V. ashei* Reed) in Alabama, Florida, and Mississippi. It was also found in wild *V. australe* in North Carolina and in wild growth of *V. ashei* in west Florida.

The disease is caused by a fungus of the *Physalospora* type that produces both perithecia and 2 forms of pycnidia (macro and micro) in infected cortex tissues. It is probably indigenous to southeastern United States, as it has been observed in wild blueberry bushes growing not only adjacent to cultivated blueberry fields but in localities several miles distant from plantings. Cankers have been seen in wild bushes in North Carolina of size and character that would indicate infection antedating the establishment of the pioneer cultivated fields in the State. The older blueberry fields in North Carolina, nearly all of which now show more or less canker, were planted with nursery stock grown in New Jersey, a section where the canker is unknown; therefore, it is evident that the disease, so prevalent in North Carolina, has spread to cultivated fields from infection foci in wild bushes.

The rabbit-eye blueberry industry in west Florida was established 30 years or more ago from seedling plants taken from wild growth along river courses of Florida and Alabama, and probably diseased plants were introduced into cultivated plantings at that time. Some canker is now present in most of those cultivated fields, varying from an occasional infected bush in some fields to as much as 50 per cent infection in others. All degrees of susceptibility, from near-immunity to complete susceptibility, are manifest in those seedling plantings. The primary shoots of very susceptible bushes show almost solid canker development from the ground line to a height of 3 or 4 feet. In spite of the extensive cankers, no substantial mortality of bushes has occurred, although many become weakened and make poor growth.

The pathogen gains entrance through the unbroken bark, probably through lenticels of current-year shoots. The points of entrance become evident in late summer or early fall as reddish, broadly conical swellings. Pycnidia usually develop on the surface of these conical growths and the bark may or may not crack the first season (Fig. 1, A). Later symptoms vary considerably, depending upon the host variety involved. The second year after infections occur on the varieties Cabot and Pioneer the small swellings lose their reddish color and conical shape, enlarge somewhat, and become fissured. The fungus invades the cortex tissues in all directions from points of infection and this newly invaded bark tissue swells, the surface becomes uneven, resembling blisters, and turns light gray (Fig. 1, B). The fungus not only invades the cortex and cambium but also penetrates slightly into the xylem, causing some discoloration but no extensive necrosis of the wood. By the end of the second year or the beginning of the third the older portions of the cankers are rough, black, and deeply fissured (Fig. 1, C). The result is girdling of the shoots, the parts above becoming unfruitful and finally dying. For some unexplained reason cankers first appear on the side of shoots directly exposed to the sunrays. This feature is well brought out in figure 2, A and B.

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[1] The writers are indebted to Dr. W. W. Diehl for assistance in identifying the fungus, to Miss Edith Cash for the Latin translation of the technical diagnosis, and to Mr. G. A. Meckstroth for certain observations and for numerous specimens.

[2] The disease was called to the attention of the writers by Mr. C. A. Doehlert, New Jersey Agricultural Experiment Station, who collected specimens near Magnolia, North Carolina, in December, 1938.

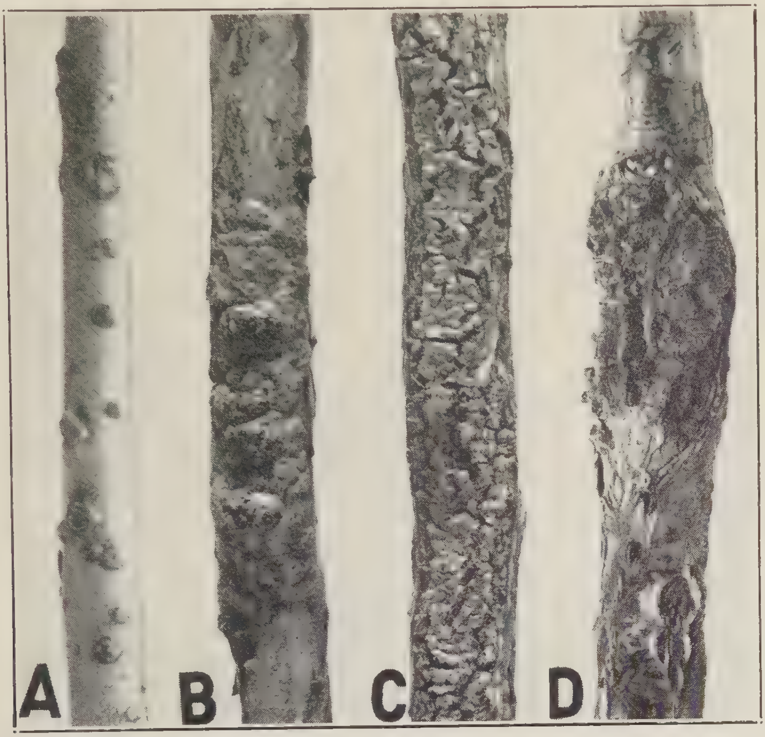

FIG. 1. Blueberry cane canker caused by *Physalospora corticis*. A. Early stage of canker infection on current year's wood. ×1½. B. Active canker about 3 years old showing "blister stage." The surface of the bark is uneven and grayish and fissures have begun to appear. ×1½. C. An old, deeply fissured canker. ×1. D. Typical callus formation around a shoot wound artificially inoculated with the canker fungus. ×2.

The cortex reaction to the fungus in some other blueberry varieties studied is less pronounced than in Cabot and Pioneer. The cankers may not be conspicuous but, instead, may show as only slightly swollen, uneven, grayish areas, with little or no cracking of the bark. The injury to the shoots of such varieties is much less severe than if cracking of the bark occurs.

VARIETAL SUSCEPTIBILITY

Varieties of the high-bush blueberry show considerable difference in their ability to resist attack by the canker fungus. The variety Cabot is doubtless the most susceptible and will perhaps be discarded in the South for this reason. Other observed varieties, in descending order of apparent susceptibility, are as follows: Pioneer, Concord, June, Stanley, Jersey, Scammell, Rancocas, and Rubel.

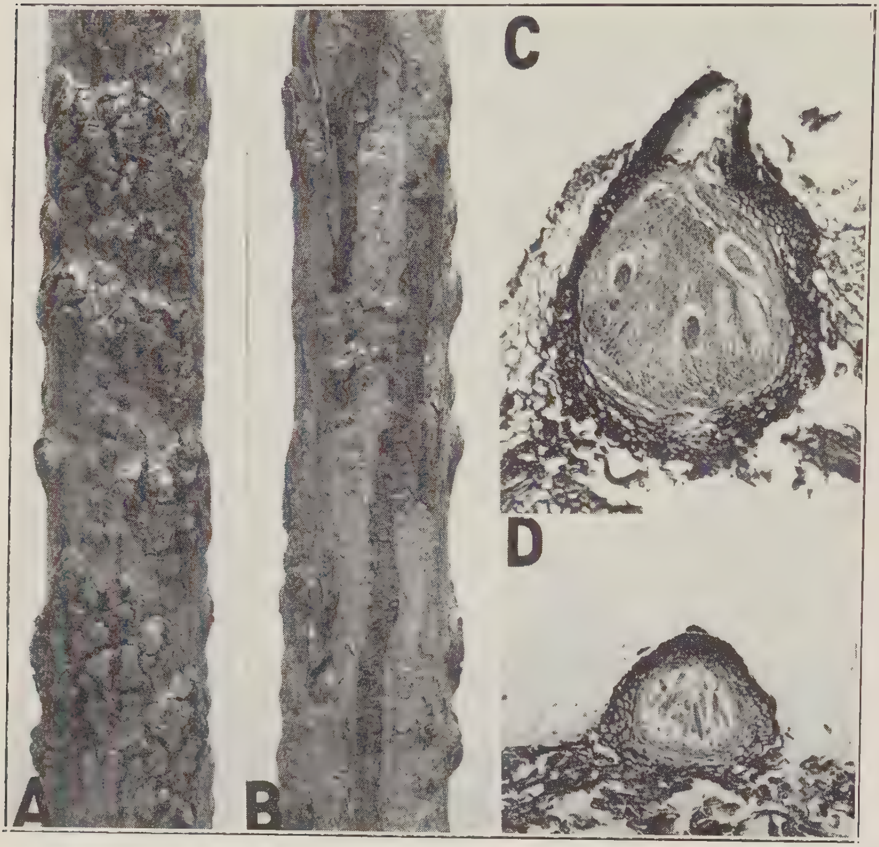

FIG. 2. A. Characteristic appearance of canker on side of a blueberry cane exposed to sunrays. B. Opposite side showing only scattered lesions and blisters. ×1. C. Perithecium of *P. corticis* showing immature asci surrounded by paraphysoid filaments. ×150. D. Pycnidium. ×150.

Named varieties of the rabbit-eye blueberry are mostly of recent selection and all have not been subjected to severe tests for susceptibility. The varieties Black Giant, Hagood, Locke, Myers, Owens, Ruby, and Scott have been examined for presence of cankers occurring naturally and they were found only on Locke.

THE FUNGUS

Pycnidial Stage

Pycnidia form in the summer, are sometimes abundant in the margin of cankers, and are frequently present on new shoot lesions (Fig. 1, A) near the termination of the first growing season after infection takes place. They are scattered, partly imbedded or superficial, black, conical to subglobose, ostiolate; those measured varied from 90–208 μ high and 60–165 μ wide (Fig. 2, D). The majority were from 105 to 140 μ by 70 to 100 μ. The pycnidial wall is carbonaceous, and rather thick, varying from 10 to 30 μ wide. The pycnospores (Fig. 3, C) are fusiform elliptical, and some are obtuse at the base and pointed at the opposite end. They are nonseptate, hyaline, averaging 35 μ long and 9 μ wide, and develop from conidiophores about 15 μ long. Another type of spore has occasionally been observed either in pycnidia or in other fruiting bodies somewhat smaller than true pycnidia. These spore structures are rod-shape, hyaline, nonseptate, about 3 to 7 μ long, and are produced on relatively long conidiophores. They are doubtless a stage in the life history of the fungus, since they form when mycelium from subcultures derived from ascospores has been used to inoculate autoclaved corn meal.

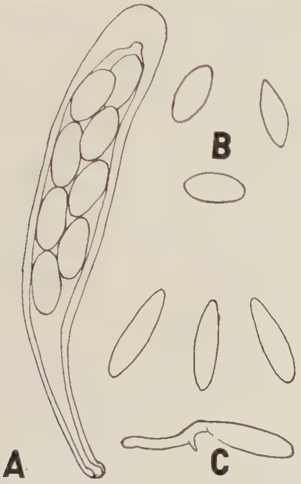

FIG. 3. *Physalospora corticis.* A. Ascus. B. Ascospores. C. Pycnospores. All about × 320.

Perithecial Stage

The perithecia are produced much more abundantly than pycnidia. Those on the older portion of cankers are usually empty, but functioning ones often can be found in late summer in the more recently invaded bark tissues. They average about 280 μ high and 200 μ wide. The typical shape is definitely conical, and the ostioles of some are distinctly pointed and drawn out into short beaks 50 to 100 μ long. Otherwise, the perithecia have about the same superficial appearance as pycnidia.

Perithecia contain at first white, erect, closely packed, short-celled, anastomosing hyphae (Fig. 2, C). Shear, Stevens, and Wilcox,[3] in discussing the character of *Botrysphaeria ribes* and *Physalospora malorum* (*obtusa*), considered similar filamentous perithecial structures to be a form of paraphyses. The asci develop from the base of the perithecium and push up through the hyphal mass. There are seldom more than a dozen asci in a perithecium, frequently only 4 to 8, separated by layers of paraphyses (Fig. 2, C).

The asci usually are clavate, contain 8 spores, and the wall is much thicker at the apex than elsewhere (Fig. 3, A). Actual liberation of ascospores has not been observed, but it is not unusual in water mounts to find asci ruptured in a characteristic manner. These asci were broken transversely immediately below the thickened cap, and the cap was pushed forward a short distance by elongation of the ascus contents. The spores did not drift apart but retained the same outline of the ascus as though they were held together by a colorless gelatinous substance or a very thin membrane, which elongated below and was attached to the inside base of the ascus.

The ascospores (Fig. 3, B) are hyaline or sometimes slightly tinted, ellipsoid to fusoid, and nonseptate. The size of those measured averaged 29.2 μ long and 11.7 μ wide.

Ascospores have been observed to germinate within 30 minutes after being mounted in tap water. The germ tubes elongated rapidly and formed unbranched hyphae approximately 120 μ long in 5 hours. At the end of a 19-hour observational period the length of the primary hyphae varied from 142 to 380 μ and no branching occurred. The spores frequently germinated while still in unruptured asci, the germ tubes passing through the ascus wall, even through the thick apex, with little or no apparent retardation in rate of growth.

CULTURAL CHARACTERISTICS

On corn-meal agar the blueberry canker fungus grew rapidly, covering the surface of agar in an ordinary Petri dish in 6 or 7 days at a temperature of 25° C. The mycelium was light to dark green, septate, coarse, and sparsely branched. Loose felty hyphae covered the surface of the colonies. No form of fructification has been observed in cultures on corn-meal agar but some pycnidia formed in flasks of autoclaved corn meal.

PROOF OF PATHOGENICITY

Pathogenicity of the *Physalospora* was demonstrated by inoculating potted blueberry plants in a greenhouse with subcultures from single ascospore isolations. The ascospores were taken from typical blueberry cankers collected in North Carolina.

Since spores could not readily be obtained from cultures, bits of agar containing mycelium of the fungus were used as the inoculating material. The inoculum was applied to wounded and nonwounded surfaces of shoots of different ages, then covered with damp cotton and wrapped with parafilm. The wounding was done by making a short longitudinal scratch in the bark. Uninoculated plants, similarly treated, served as controls.

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[3] Shear, C. L., Neil E. Stevens, and Marguerite S. Wilcox. *Botryosphaeria* and *Physalospora* on currant and apple. Jour. Agr. Res. [U.S] 28: 589–598. 1924.

In March and April, 1939, 54 inoculations were made on blueberry seedlings. Wounds were made for 25 of the inoculations, while 29 were not wounded. One- and 2-year-old shoots principally were used. At the termination of the growing season there was neither evidence of infection on any of the nonwounded shoots nor any clear indication of canker formation on the wounded ones. All except 9 of the wounded shoots, however, produced a callus formation around the wound, and a few *Physalospora* pycnidia formed in the callus tissues of some, but no swelling, unevenness, cracking or other signs characteristic of canker, known under field conditions, developed. The presence of callus growth and of pycnidia thereon was not considered a criterion of infection, and the plants were discarded. Plants similarly inoculated in 1940, as described below, were held over for observation through 1941, when it was found that a single growing season was insufficient time for development of definite signs of pathogenicity of the fungus. This suggests that the 1939 inoculated plants may have been discarded before there was time for typical symptoms to appear.

In 1940, the work was repeated in about the same manner as the previous year except that plants of the very susceptible variety Cabot were used rather than seedlings. Thirty-four inoculations were made principally in wounded succulent and 1-year-old wood. Final observations made at the end of the growing season showed results similar to those obtained the previous season, *i.e.*, unwounded plants showed no evidence of infection but all wounded shoots made the same type of callus formation as was noted in 1939.

At the close of the growing season of 1941 the plants inoculated in the spring of 1940 showed a high percentage of canker. There were typical canker signs on all wounded inoculated shoots, of which 11 were of the current season's growth, 15 were 1 year old, and 1 was 2 years old when inoculated. Of 4 nonwounded succulent shoots 1 showed canker development; of 3 nonwounded 1-year-old shoots all showed cankers. At the end of the second year the callus formation on most of the inoculated wounded shoots had enlarged and in a few cases had girdled the shoot (Fig. 1, D). The most positive indication of the pathogenic nature of the organism used for the inoculation was the extension of an infected area around the point of inoculation as shown by culturing the cortex tissues, by the grayish color, unevenness, and cracking of the bark surface, and by the presence of perithecia and pycnidia, all being characters common in newly invaded bark tissues adjacent to naturally-formed cankers. Signs of the fungus invasion extended from 2 to 5 cm. above and below the area where the inoculum was applied.

IDENTITY OF THE FUNGUS

Provided the tangled anastomosing hyphae within the perithecia and among the asci of this blueberry canker fungus can be considered of a paraphysoid nature, its morphological characteristics would then place it in the genus *Physalospora*. Species of the closely related fungus *Botryosphaeria* commonly form perithecia in groups on a stroma. Although some stromatic material is mixed with the blueberry canker tissues, the perithecia are distinctly scattered and do not necessarily grow from stromatic tissue.

The blueberry fungus is similar in some respects to *Physalospora obtusa*. Both fungi have the same type of filamentous packing around the asci. Asci and ascospores are about the same size and shape and are similar otherwise. The apical portions of asci of both are considerably thickened and both exhibit the same peculiar method of rupturing of asci back of the thickened tip. The germ tube of the blueberry fungus elongates to a considerable length before branching, a characteristic also common for *P. obtusa*. Both fungi produce two types of pycnospores—macro and micro. There are, however, clearly some differences between the 2 fungi. The mature macropycnospores of *P. obtusa* are brown, while those of the blueberry fungus are hyaline and of a distinctly different size and shape. When grown in Petri dishes on corn-meal agar, under identical conditions, the apple fungus makes colonies twice the diameter of those of the blueberry fungus. The blueberry *Physalospora* produces perthecia more abundantly than pycnidia, while the pycnidial stage of *P. obtusa* is the more common. Another difference between the 2 fungi is the reaction when they are inoculated into apple fruits. *P. obtusa* produces an extensive and rapid decay in apples followed by the production of *Sphaeropsis* pycnidia. When the blueberry canker fungus was inoculated into apple fruits no decay developed. Therefore, although the 2 fungi are similar in some respects, they are so different in others that they are considered to be 2 different and separate identities.

Since the writers found no record in literature of a *Physalospora* pathogenic in any Ericaceous plant it is thought that the blueberry canker fungus has not been previously described and named. Since the fungus insofar as is known at present inhabits principally the cortical tissues of canes and shoots, a name to indicate that characteristic is being applied with the following description:

Physalospora corticis n. sp.

Perithecia mostly scattered, partly imbedded, black, globose to conical, ostiolate, about 200–350 μ high and 170 to 210 μ wide; asci 8-spored, clavate, wall thick especially at the apex, hyaline, 112–180 μ long and 27–32 μ wide; paraphyses erect, anastomosing, filamentous; ascospores hyaline to slightly tinted, ellipsoid to fusoid, continuous, 24–37 μ long and 9.6 to 16 μ wide.

Pycnidia scattered, black, sub-globose to conical, wall thick, 90–208 μ high and 60–165 μ wide. Pycnospores hyaline, non-septate, fusiform elliptical, 27–45 μ long and 8–12 μ wide and produced on conidiospores about 15 μ long.

Small hyaline non-septate globose to rod-shape micropycnospores about 3–7 μ long, sometimes present with pycnospores or in separate pycnidia.

Parasitic in stems and shoots of blueberries (*Vaccinium australe* Small and *V. ashei* Reed), causing cankers, in Alabama, Florida, Georgia, North Carolina, and Mississippi.

Perithecia plerumque conspersa, partim immersa, atra, globosa vel conica, ostiolata, 200–350 μ alta, 170–210 μ lata; asci octospori, clavati, tunica praesertim apice incrassata, hyalini, 112–180 μ longi, 27–32 μ lata; paraphyses rectae, anastomosantes, filamentosae;

ascosporae hyalinae vel pallide coloratae, ellipsoideae vel fusoideae, continuae, 24–37 μ longae, 9.6–16 μ latae.

Pycnidia conspersa, atra, subglobosa vel conica, pariete crasso, 90–208 μ alta, 60–165 μ lata; pycnosporae hyalinae, continuae, fusiform ellipticae, 27–45 μ longae, 8–12 μ latae; e conidiophoris circa 15 μ longis productae; micropycnosporae parvae, hyalinae, continuae, globosae vel bacilliformes, circa 3–7 μ longae, pycnosporis associatae vel pycnidiis separatis interdum praesentes.

Hab. cancros in caulibus surculisque Vacciniorum producens, in Alabama, Florida, Georgia, North Carolina, et Mississippi.

A type specimen of the fungus has been deposited in the Mycological Collections of the Bureau of Plant Industry, U. S. Department of Agriculture, at Washington, D. C., under No. 71,375. This specimen was collected in a North Carolina cultivated field of *Vaccinium australe.*

CONTROL

Spraying blueberry bushes with Bordeaux mixture and with lime-sulphur solution in both winter and summer has given inadequate control of the disease.

Growers have attempted to hold the disease under control by pruning out all visible cankers, even taking out badly infected bushes, but this practice has proved of little value. A great majority of the conspicuous cankers can be removed, especially if they are on the secondary shoots, but it is difficult to train workmen to recognize the more incipient stages where evidence of the disease is in the form of small reddish conical swellings or unevenness of the bark.

The most promising lines of attack are the planting of disease-free plants and the use of resistant varieties. Selection of disease-free plants for transplanting will not always guarantee freedom from the disease in some southern sections, as the wild blueberries in nearby swamps and forests are sometimes infected and the disease may spread from them to cultivated fields. Therefore, at present, the best recommendations for combating blueberry canker is to grow only those varieties known to be resistant. Cabot and Pioneer are very susceptible varieties, and, if planted in the South, they should not be near infected fields or in localities where there is wild-blueberry growth.

SUMMARY

This paper discusses a fungus disease of considerable economic importance prevalent in southeastern United States, causing extensive and damaging cankers on blueberry stems and shoots.

The disease has been observed in cultivated plantings in Alabama, Florida, Georgia, Mississippi, and North Carolina, and in wild blueberry plants in Florida and North Carolina. Evidence indicates that the causative fungus is indigenous on wild blueberries in the South and has spread from them to cultivated plantings.

The fungus is thought to be an undescribed species of *Physalospora* and the name **P. corticis** n. sp. is proposed.

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PHYTOMONAS POINSETTIAE N. SP., THE CAUSE OF A BACTERIAL DISEASE OF POINSETTIA

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The poinsettia, *Euphorbia pulcherrima,* probably is the most important potted plant for the Christmas market. Heretofore, the most serious disease of this plant has been a stem rot of rooted cuttings and of young plants caused by a species of *Rhizoctonia.* A new and much more destructive disease, caused by a bacterium, has been under investigation since July, 1941. The first recorded outbreaks of this disease occurred in two widely separated greenhouse establishments in New Jersey during the summer of 1941; by November, it was known to occur in Maryland, Pennsylvania and New York. Preliminary studies of this disease were made by the junior author and his assistant (7) who also first isolated the bacterium herein conclusively proved to be the specific etiological agent.

DESCRIPTION OF THE DISEASE

Symptoms are readily visible in all above-ground plant parts. Longitudinal, water-soaked streaks, usually on one side of green stems, are the most characteristic symptoms. Such streaks may continue up through the leaf petioles, resulting in spotting or blotching of the leaves and in complete defoliation. The streaking may continue downward into the woody stem, detectable only by a bark incision. A yellowing of the cortex and browning of the vascular system is revealed when the epidermis is removed above the water-soaked lesions on green stems. In advanced stages, the stems crack open in a very unsightly manner, and bend down sharply towards the unaffected side.

Glistening, golden-brown masses of bacteria occasionally ooze from the ruptured stems and from leaf lesions. Microscopic sections of invaded stems show bacteria in profusion in the phloem.

Cuttings obtained from infected stock plants fail to develop into desirable plants. In one greenhouse these, as well as the stock plants, were a total loss within a month after the disease was first observed.

ISOLATION AND PROOF OF PATHOGENICITY OF THE CAUSAL ORGANISM

The causal organism was first isolated by the junior writer from diseased stock plants on July 25, 1941. The surface of young green stems showing typical longitudinal streaks was wiped with 95 per cent alcohol and the epidermis peeled off. Dilution plates prepared from the yellowed cortical tissues thus exposed yielded a single kind of bacterial colony. Early in August, isolates from these plates were inoculated by means of needle pricks

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into 12 young shoots on 4 vigorous plants just above the leaf axils. Within 3 weeks unmistakable lesions appeared on the stems. In some instances these had progressed sufficiently to involve the leaves and stem tips above the inoculated areas. Within 5 weeks after inoculation, the stems cracked open and the exposed tissues became brown and dry in much the same way as the naturally infected stems. Control plants receiving only needle pricks and sterile nutrient broth remained healthy throughout the course of the work. Bacteria, reisolated from these artificially infected plants, were morphologically similar to those inoculated.

Two of the original isolates (CP 1 and CP 2)[3] were sent to W. H. Burkholder, Cornell University, who, on September 5, 1941, succeeded in causing typical symptoms by needle-pricking the young stems of potted poinsettias with these two isolates. Uninoculated, but wounded, controls remained healthy. Bacteria (CP 3 and CP 5),[3] morphologically similar to those inoculated, were isolated November 25, 1941, by the senior writer from lesions of these artificially infected plants.

On October 15, 1941, the senior writer isolated similar bacteria (CP 13 and CP 14)[3] from lesions of a naturally infected diseased poinsettia that had been forwarded by W. H. Burkholder. Ten isolates from this plant were inoculated into wounds made with the tip of a sharp, sterilized scalpel on the young stems and petioles of 10 healthy poinsettias. Characteristic symptoms appeared about the sites of inoculation on all 10 plants within 4 weeks; within 6 weeks, the stems had cracked open. Bacteria (CP 20 and CP 21)[3] resembling those that had been applied as inoculum were isolated from the advancing margins of the lesions from 3 to 20 weeks after inoculation. Wounded, noninoculated control plants remained healthy throughout these experiments.

Another series of isolations (CP 27, 31, 35, 37, 39 and 41)[3] was made by the junior writer on November 10, 1941, from diseased stock plants originally obtained from the greenhouse, where the disease was first noted in July, but had been held over in the interim in both the plant pathology and ornamental horticulture greenhouses at New Brunswick. Isolations were attempted from 16 stems on 6 stock plants nearly 4 months after these plants first showed typical symptoms. These were made from brown longitudinal streaks beneath unruptured stems, from brown subsurface lesions, just beyond the ruptured stem areas, and from deep discolored lesions originating at stem ruptures. In nearly all cases, a species of bacterium, morphologically resembling that obtained in the original isolations was recovered. Three isolates were inoculated November 17, 1941, on 6 stems in a manner similar to that already described. Well-defined lesions did not appear on these stems until 5 weeks after inoculation. The delay in appearance of symptoms may have been due to the lower greenhouse temperatures prevailing at that time. Adequate controls remained healthy. Bacteria similar to those inoculated were isolated from typical lesions.

[3] The isolates designated in parentheses are the 14 upon which is based the following description of the pathogen.

CONTROL SUGGESTIONS

It is suggested that stock plants known to harbor the pathogen be discarded, even though the new shoots used for propagation appear normal. Rooted cuttings and young plants obtained from questionable sources should be kept separate from those known to come from healthy stock. Overhead watering and syringing should be minimized to avoid spreading the bacteria, which ooze from infected stems and leaves. It may be of interest to note that healthy plants were grown successfully in the greenhouse involved in the July 25th outbreak, following complete removal and burning of all diseased plants, steam sterilization of the soil and disinfection of the benches, pots and other greenhouse apparatus. It is not definitely known whether these measures were entirely responsible for the apparent control.

DESCRIPTION OF THE PATHOGEN

A representative group of isolates[4] from naturally and artificially infected poinsettias was studied in detail by the senior writer. The technique used in this study is that described in the *Manual of Methods for Pure Culture Study of Bacteria* (9) and in the *Manual of Dehydrated Culture Media and Reagents* (4). Where the procedure used is not exactly as described in these publications, appropriate particulars of the technique are given or other sources are cited.

Except where indicated otherwise, incubation of cultures was carried out at 27° C. The names of colors used in the descriptions follow those suggested by Ridgway (8). In all cases, 14 isolates[4] were studied simultaneously and, unless stated otherwise, identical reactions were obtained for all the isolates.

Morphology and Staining Reactions

Highly pleomorphic: straight rods, comma-shape, curved, coccoid, clavate, wedge-shape, and bizarre involution forms, occurring singly and in palisade arrangement. Barred and granule-bearing forms common. Endospores not formed. Capsules formed in some sugar-containing media. Motile by one, rarely two, polar or lateral flagellum. Size very variable: $0.2\text{–}0.8 \times 0.5\text{–}8.5\ \mu$, averaging $0.3\text{–}0.6 \times 1.0\text{–}3.0\ \mu$. Gram-positive, becoming Gram-variable, as shown by Hucker's modification of Gram's stain. The Gram-negative cells in old cultures often contain Gram-positive granules; these granules are also disclosed by staining with Loeffler's alkaline methylene blue. Not acid-fast by the Ziehl-Nielsen method. Stained readily by carbol-fuchsin and malachite-green. Cells are not stained by Congo red or nigrosin. Flagella of cells grown on moist beef extract agar slants of pH 6.9 are stained easily by Gray's method.

Cultural Characteristics

Colonies on Beef-extract Agar (0.3 per cent beef extract, 0.5 per cent peptone, 1.5 per cent agar; pH 6.9). Surface colonies are round, slightly convex, ranging from 0.1 to 1.0 mm. and averaging 0.2 to 0.5 mm. in diameter; deep colonies are ellipsoidal. Amorphous or finely granular structure detectable at a magnification of 440 ×; edges are entire at 100 ×. Smooth, non-viscid, moist, colorless, almost transparent.

Colonies on Potato-dextrose Agar (Bacto; pH 5.6). Surface colonies are round, slightly convex, ranging from 0.5 to 4.0 mm. in diameter; deep colonies are ellipsoidal, $0.1\text{–}0.3 \times 0.3\text{–}0.8$ mm. Finely granular structure evident at 440 ×; edges entire at 100 ×. Smooth, glistening, non-viscid, mucoid, moist, salmon color becoming ochraceous salmon.

Colonies on Tryptose-phosphate Agar (Bacto tryptose phosphate broth plus 1.5 per cent agar; pH 7.3). Surface colonies are round, convex, 4 mm. or more in diameter; deep colonies are ellipsoidal averaging 0.4 × 1.5 mm. Finely granular structure visible at 440 ×; edges entire at 100 ×. Glistening, smooth, mucoid, moist, frequently becoming very viscid, carrot-red or flesh color; deep colonies are colorless.

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[4] See footnote 3.

Colonies on Blood Agar (Bacto-blood agar base plus 5 per cent sterile, defibrinated rabbit blood; pH 6.9). Surface colonies round, convex, under 1 mm. in diameter. Structure amorphous at 440 ×; edges entire at 100 ×. Glistening, moist, slightly viscid, white or pale-salmon. No visible action on blood.

Colonies on Tellurite-blood Agar (Bacto-dextrose-proteose No. 3 agar plus 5, 8 or 10 per cent bacto-tellurite-blood solution; pH 7.4). Surface colonies round, convex, 0.3 to 2.5 mm. in diameter. Amorphous structure, entire edges, smooth, glistening. Light to deep neutral-gray, rarely pale or pallid neutral-gray. Tellurite reduced.

Beef-extract Agar Slants (pH 6.9). Scanty, filiform, glistening, non-viscid, colorless, almost transparent.

Potato-dextrose Agar Slants. (Bacto; pH 5.6). Moderate, filiform, glistening, non-viscid, salmon or flesh color.

Tryptose-phosphate Agar Slants (Bacto-tryptose-phosphate broth plus 1.5 per cent agar; pH 7.3). Abundant, filiform, later spreading, raised, glistening, mucoid, often becoming extremely viscid, orange-pink to geranium-pink.

Potato Slants. Abundant, spreading, not raised, glistening, moist, becoming butyrous or somewhat viscid, orange, Mikado orange, deep chrome- or capucine-yellow. Potato distinctly browned in ten days.

Loeffler's Blood-serum Slants (Bacto; pH 7.2). Moderate, filiform or spreading, glistening, non-viscid, carnelian-red. Medium liquefied in 3 to 10 days.

Agar-shake Cultures (2 per cent tryptose, 0.5 per cent yeast extract, 1.5 per cent agar). Growth only under distinctly aerobic conditions in upper 1 to 2 mm.

Thioglycollate Broth (BBL thioglycollate medium (1) with dextrose and Eh indicator; pH 7.4). Flocculent pellicle and dense, viscid growth in upper 2 or 3 mm. and no trace of growth in anaerobic medium below, thus indicating a distinct partiality for aerobic conditions.

Litmus Milk (pH 7.0). Only very slight acidity or no visible change for 1 to 2 weeks after inoculation; then, soft coagulation, reduction of litmus, followed by a rapid, complete peptonization.

Lead Acetate Agar (Bacto; pH 6.6). Moderate auburn surface growth, extending 1 to 2 cm. along line of stab as a scanty beaded growth. Medium not browned, except slightly after 3 or 4 weeks; it is questionable whether this slow, scanty browning should be interpreted as a definite production of hydrogen sulphide, especially since hydrogen sulphide cannot be demonstrated by means of lead acetate paper suspended in tryptone-broth cultures, even after 10 weeks' incubation.

Beef-extract Broth (0.3 per cent beef extract; 0.5 per cent peptone; pH 6.9). Turbid within 24 hours, later viscid, pale-salmon sediment; no pellicle or ring formed.

Tryptose-phosphate Broth (Bacto; pH 7.3). Turbid within 24 hours, later, abundant flesh-pink, viscid sediment; sometimes, ring and pellicle.

Tryptone Water (1 per cent Bacto tryptone; pH 7.0). Turbid within 24 hours, later, viscid sediment; no pellicle or ring.

Range of Temperatures for Growth. Active cultures were transferred to small tubes of Bacto-tryptose phosphate broth of pH 7.3 previously brought to the given temperature, incubated there, and examined daily for the definite turbidity that indicates growth. After 24 hours, growth occurs between 15 and 36° C. and, after 48 hours, between 7 and 12° C. No growth after a week from 0.5 to 5° C. or from 37 to 50° C.

Carbon Metabolism

Utilization of Sodium Citrate as Sole Carbon Source. Koser's citrate medium (Bacto; pH 6.7) does not support growth.

Utilization of Glucose as Sole Carbon Source. A synthetic medium consisting of 0.1 per cent $NH_4H_2PO_4$, 0.02 per cent KCl, 0.02 per cent $MgSO_4 \cdot 7H_2O$, 1.0 per cent glucose, pH 6.4, does not support growth, even after 8 weeks.

Action on Cottonseed Oil. No lipolytic activity revealed by spirit-blue cottonseed-oil-agar technique (10, 11).

Voges-Proskauer and Methyl-red Tests (Bacto M.R.–V.P. medium; pH 6.9). No acetylmethylcarbinol can be demonstrated in 72-hour-old cultures by the Werkman (12) procedure. Methyl-red test is also negative.

Sugar Fermentations. The ability to ferment carbohydrates, alcohols, and glycosides was determined by means of Durham tubes with a medium consisting of 0.3 per cent yeast extract, 0.5 per cent peptone, 0.5 or 1.0 per cent of the sugar, pH 7.5, with either 1 per cent Andrade's indicator or 0.0018 per cent phenol red to demonstrate acid production. As is indicated below, acid is produced from many sugars; gas is not formed.

No acid produced after three weeks: Rhamnose,[5] fucose,[6] inulin,[5] glycogen,[6] mannitol,[5] dulcitol,[5] sorbitol[6] inositol[6] (except isolates CP 27, 31, and 41, which ferment inositol slowly).

Weak, slow acid production: Arabinose[5] (9 days), xylose[5] (9 days), lactose[6] (2 to 3 weeks; acid production from lactose cannot be demonstrated, even after 6 weeks, when Andrade's indicator is used), trehalose[6] (12 days), dextrin[5] (8 days), adonitol[6] (9 days).

Moderate to abundant acid production within 5 days: Glucose[6] (when Andrade's indicator is used, there is demonstrable only a slow and variable production of acid from glucose), fructose,[5] mannose,[5] galactose,[5] sucrose,[5] maltose,[5] cellobiose,[6] melibiose,[6] raffinose,[6] glycerol,[5] erythritol,[6] salicin,[5] amygdalin.[6]

Hydrolysis of Starch (0.3 per cent yeast extract, 0.5 per cent tryptone, 0.5 per cent "soluble" starch). Complete hydrolysis after 11 days, but not after 4 days, as shown when portions of the cultures are tested with Lugol's iodine solution on a spot plate.

Action on Cellulose. No visible action on strips of filter paper immersed in beef-extract broth cultures for 8 weeks.

Nitrogen Metabolism

Indole Production. No indole formed in 1 per cent Bacto tryptone of pH 7.0 after 3, 6, 9 or 15 days, as determined by the Ehrlich-Böhme procedure.

Reduction of Nitrates. No reduction of 0.1 per cent $NaNO_3$ in beef-extract broth of pH 7.0 in six days, as shown by the sulphanilic acid-α-naphthylamine test.

Urea Hydrolysis. No hydrolysis of a 1 per cent aqueous-urea solution by heavy suspensions of 3-day-old cells during an incubation of 5 hours at 37° C., as determined by a negative Nessler test for ammonia. This procedure can demonstrate ureolysis by *Bacillus pasteurii* and ureolytic micrococci after an incubation period of only 1 hour at 37° C.[7]

Utilization of Uric Acid as Sole Nitrogen Source. Koser's uric acid-glycerol medium (6) does not support growth.

Utilization of Asparagin as Sole Source of Both Carbon and Nitrogen. A synthetic medium consisting of 0.1 per cent KH_2PO_4, 0.02 per cent KCl, 0.02 per cent $MgSO_4 \cdot 7H_2O$, 0.5 per cent asparagin, pH 6.4, does not support growth.

Action on Gelatin (0.3 per cent beef extract, 0.5 per cent peptone, 12 per cent Bacto gelatin; pH 6.6). Definite crateriform liquefaction in 3 days at 23° C.; later, stratiform liquefaction extending down 2 to 4 cm in 2 weeks at 23° C.

Hydrolysis of Sodium Hippurate (0.3 per cent yeast extract, 0.5 per cent tryptone, 0.5 per cent sodium hippurate). After 7 days' growth, benzoic acid was sought by adding either 1 volume of 7 per cent $FeCl_3$ or of 50 per cent H_2SO_4 to 4 volumes of culture and looking for a precipitate of ferric benzoate or of benzoic acid, respectively. No hydrolysis of sodium hippurate is demonstrated by this technique, although a known hippurate-splitting species, *Streptococcus agalactiae*, does bring about demonstrable hydrolysis.

TAXONOMY OF THE PATHOGEN

The monotrichic phytopathogenic bacteria are now included in the genus *Phytomonas* Bergey *et al.* The poinsettia pathogen, described above, is unlike any now included in that genus in the fifth edition of *Bergey's Manual of Determinative Bacteriology* (2); hence, the name **Phytomonas poinsettiae** n. sp., is proposed.

Burkholder (2, 3) and others have discussed the heterogeneous nature of the genus *Phytomonas* Bergey *et al.*, and have shown that, actually, it consists of several unrelated groups of bacteria. It seems likely, on the basis of characteristic morphological and cultural properties, that this poinsettia pathogen is closely related to those Gram-positive, phytopathogenic bacteria, now (2) in Appendix II of the genus *Phytomonas* Bergey *et al.*, some of which have been more correctly included (5, 8a, 11) in the genus *Corynebacterium* Lehmann and Neumann. When the latter genus is emended to include motile forms, this species might be included as *Corynebacterium poinsettiae.*

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[5] Andrade's fuchsin indicator was used for demonstrating acid production.
[6] Phenol red was used for indicating production of acid.
[7] Starr, Mortimer P. Unpublished observations, 1940.

SUMMARY

A serious disease of the poinsettia, *Euphorbia pulcherrima*, is described. A bacterium, proved beyond all question to be the specific etiological agent of this disease, is described in detail. Since the causal agent is different from all previously reported species of phytopathogenic bacteria, the name **Phytomonas poinsettiae** n. sp., is proposed for this pathogen, and it is recommended that this species be included in the *Corynebacterium* group of plant pathogenic bacteria.

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BORON DEFICIENCY IN PEAR TREES

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Boron has been applied to pear orchards in an attempt to correct various troubles of the fruit and tree. A cracking and dieback of pear in New South Wales (1, 2) has been corrected by soil applications of borax. Noble (10) reports a blossom and twig blight in the same country, which appeared to be associated with boron deficiency. A roughening and cracking of Bosc pears responded to boron treatments in Tasmania (3), but it was pointed out that ''crinkle'' was not affected by the treatment. McLarty (9) illustrated boron deficiency symptoms on unnamed pear varieties in British Columbia, but Eastham (6) reported that drought spot of Bosc pears was unaffected by boron applications in the western part of the Province. One report from Washington (13) suggested that the incidence of a hard end and cork-spot complex of Anjou and Bartlett pears may have been reduced by boron treatments.

BORON TREATMENTS ON PEAR TREES IN OREGON

A type of pitting on Bosc pear fruits was noticed in 1937 at Hood River, Oregon. The pitting differed from that caused by the stony-pit virus (8), although the two were often associated on the same tree. Following 1937, the Bosc trouble increased in severity; fruit cracking appeared, less leaf surface developed, production decreased, and considerable dieback was evident by 1940. A similar trouble on Bartlett pears also was seen in 1937, but, following boron applications to this orchard by the grower, the trouble did not reappear. The same malady was again found on this variety in a small irrigated orchard at The Dalles, Oregon, in 1938, and at Hood River in 1941.

In 1940 a small uniform block of Bartlett pear trees was selected at The Dalles for boron treatment. These trees were approximately 12 years of age, and had produced only pitted fruits in 1939. Twelve ounces of granular borax was applied to the soil about the trees on February 9, 1940. Fruit failed to set in 1940 because of a lack of proper pollination and late frosts. The effect of the treatment was apparent in the 1941 crop, however, and results as recorded on August 6 of that year are given in table 1.

It will be observed (Table 1) that boron corrected the pitted condition of the fruit. The higher average yield per tree of the treated trees appeared to be caused by elimination of a blighting of blossoms prevalent in the plot of untreated trees.

Individual trees of the Bosc variety at the Hood River Experiment Station were selected for treatment in 1941. The trees were of different ages and located in various parts of the pear orchard. It had been observed in previous years that they produced pitted or cracked fruits and that dieback of the twigs had developed on several of them. One pound of granular

TABLE 1.—*Effect of boron applications on Bartlett pear pitting*

Type of treatment	Trees in plot	Sound fruits 1941	Pitted fruits 1941	Average fruits per tree, 1941
	Number	*Number*	*Number*	*Number*
Checks—no boron	10	201	472	67.3
12 oz. borax per tree, Feb. 9, 1940	14	1255	0	89.6

borax was applied to the soil about each tree on March 7, 1941. The amount of pitted fruit produced the season before and the season immediately after treatment is shown in table 2.

TABLE 2.—*Effect of boron applications on Bosc pear pitting and cracking*

Type of treatment	Trees in plot	1940		1941		Average fruits per tree, 1941
		Sound fruits	Pitted fruits	Sound fruits	Pitted fruits	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Check trees—no boron	6	1186	115	1652	327[a]	330
1 lb. borax per tree, Mar. 7, 1941	8	220	942	3424	0	428

[a] Records taken Sept. 1, 1941.

It is evident that boron applications eliminated the fruit pitting. Half of the treated Bosc trees bore fruit with stony-pit symptoms both before and after treatment, thus substantiating former experience that stony pit is not responsive to boron treatments (8).

SYMPTOMS OF BORON DEFICIENCY ON BOSC AND BARTLETT PEARS

Observations during the past 5 years indicate that pear trees are less sensitive to boron deficiency than most apple varieties. From the limited material available for observation it also appears that more variation in the symptoms may be expected among pear varieties. Of the 3 leading commercial varieties grown in Oregon, the susceptibility to boron deficiency in descending order appears to be: 1. Bosc, 2. Bartlett, and 3. Anjou. The specific symptoms of the deficiency on the first 2 varieties may be summarized as follows:

On the Fruit. Circular to angular, blunt bottomed, shallow depressions (4–10 mm. in diameter by 2 mm. or less in depth) may be present on the fruit surface. They are more abundant toward the calyx end and often merge into an extended area resembling certain forms of the hard- or black-end disease. The color of the pitted surface and of the tissue within and immediately surrounding each pit, often remains darker green than adjacent tissue. A small central core of corky tissue underlies each depression and tissue adjacent to this cork rapidly becomes brown upon exposure to air.

The calyx region may be pointed, sunken, or somewhat lopsided, but usually has the general appearance of the disease known as hard end. (This feature is less evident in the Bosc variety.) The calyx tissue is invariably hard when cut. Masses of brown, corky cells are generally present under the calyx lobes and may extend well into the core area. Mild to severe cracking of the fruit may accompany the shallow pitting of Bosc pears. The form of the pits and the corky calyx region serve to distinguish this trouble from most other types of pitting found on pear fruits (Fig. 1).

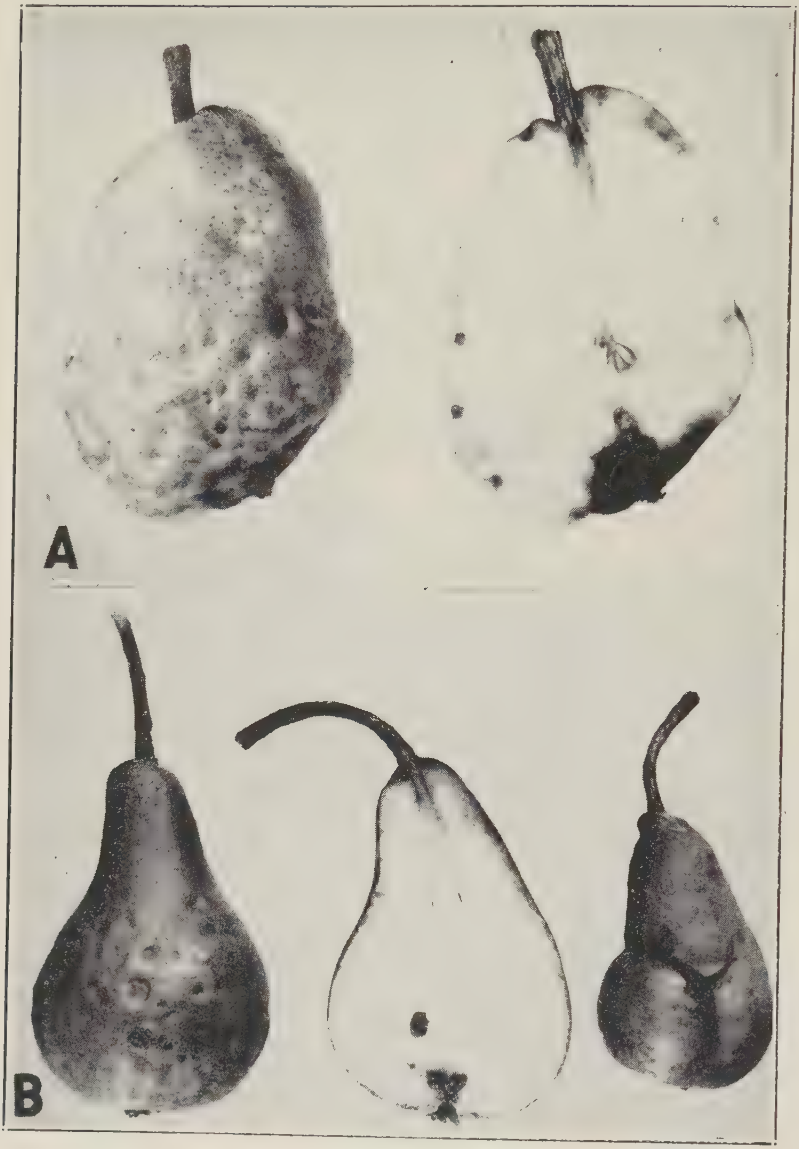

FIG. 1. Boron deficiency symptoms on Bartlett pear fruits (*A*) and Bosc pear fruits (*B*).

On the Tree. Irregular, superficial, dark or light colored bark cankers with slightly raised, uneven borders may appear on younger branches. These borders tend to give a somewhat flattened appearance to the affected parts. Fewer basal leaves develop on diseased twigs; eventually 1 or 2 sets of terminal, dwarfed leaves may form the total leaf surface. A dieback of the twigs follows and the tree gradually dies, unless the deficiency is corrected. Symptoms on the tree may develop without fruit symptoms, or only poorly defined symptoms may accompany the fruit pitting.

STONY PIT IN RELATION TO BORON

More experience is needed to distinguish between the true boron-deficiency and stony-pit symptoms on different pear varieties. Cork or crinkle of pears failed to respond to boron treatments in Tasmania (5), but a roughened and cracked condition of the fruits was corrected (3). Since stony pit is known to occur in British Columbia on Bosc pears, Eastham (6) may have been dealing with the virus trouble. Stony pit often is called drought spot by fruit growers. An illustration of the internal cork, bitter pit, and crinkle of Bosc pears as they occur in Australasia (4, Fig. 4) leaves little doubt that they are in reality stony pit. The varieties listed as susceptible to cork by Carne and Martin (5) are of interest also, since several of them have been found to be susceptible to stony pit in Oregon. Bartlett has been proved to be a symptomless carrier of the stony pit virus at Hood River.

Insufficient material of Anjou pears showing boron-deficiency symptoms has been examined by the writer to distinguish boron deficiency with certainty from stony pit. From the limited material observed, however, it appears that fruit symptoms for both troubles may be quite similar, and this may be true for other varieties, as well. Sufficient evidence has accumulated to warrant the statement that boron has no direct influence in correcting the stony pit disease. Since stony pit symptoms may become partly masked, or vary in intensity during different seasons on the same tree, sufficient trees should be observed to evaluate the variation in these symptoms.

BLACK END AND BORON TREATMENTS

The disease of pears commonly known as hard end or black end is usually attributed to the grafting of commercial varieties on oriental rootstocks (7). A report from Washington (13) suggested that boron treatments may have reduced the incidence of this and a cork spot of Anjou and Bartlett pears. The symptoms of cork or drought spot in Anjou pears were previously described from Washington (11, 12). They bear some similarity to those of stony pit as it has been observed on Anjou pears in Washington and Oregon.

There is some evidence that several hard-end or pit conditions of unknown origin may appear in pear fruits. Some of these may occasionally respond to boron treatments. The types of hard- or black-end attributed to stock and cion relations, however, have not been corrected with boron applications. A well kept Anjou orchard that had produced considerable worthless fruit affected with black end in past years was selected for treatment at Hood River. The trees were over 25 years of age, remarkably uniform in appearance, and on *Pyrus serotina* rootstocks. Besides the 3 test plots, an additional 5-acre tract treated by the grower was kept under observation. The experimental plots consisted of check trees left untreated, trees sprayed with borax (25 pounds in 300 gallons of water) at the delayed dormant period, and trees that received borax as a soil application in early spring. The results of these treatments are given in table 3.

The fruit was severely affected by black end in 1938, only slightly affected in 1939, and moderately so in 1940. Development of black end was not correlated with boron treatments, and varied considerably from year to year. Analyses of selected terminal leaves indicate that boron passed from the

TABLE 3.—*Effect of boron treatments on black end of Anjou pears*

Type of treatment 1939	Trees in plot	Black end infected fruit			Boron in leaves, August 1939[a]
		1938	1939	1940	
	Number	*Per cent*	*Per cent*	*Per cent*	*p.p.m.*
Check trees—no boron	27	100	1.7	90	20.4
1 lb. borax per tree on soil, Mar. 7	11	100	1.8	95.9	31.3
Borax spray—(25–300) on trees, Mar. 24	24	100	1.5	96.2	27.7

[a] Analyses by L. P. Batjer. Dr. Batjer stated that greater differences probably could be expected from analyses of fruit samples.

roots to the terminals through the cion unions on these trees growing on roots of a species known to be associated with the black end condition.

SUMMARY

Boron applications to pear trees corrected a condition characterized by pitting of fruits, especially near the calyx end, and by superficial cankers on younger branches. A dieback of twigs follows, and the tree gradually dies, unless the deficiency is corrected.

The symptoms of boron deficiency on fruit are similar to those of stony pit, a virus disease.

It is suggested that the disease of pear variously termed crinkle, bitter pit, and internal cork in Australasia is the same as stony pit.

The black end of pear fruits, attributed to stock and cion relations, was not corrected by applications of boron.

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VARIATION IN REACTION OF ANTHONY OATS TO STEM RUST, PUCCINIA GRAMINIS AVENAE[1]

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In July, 1939, numerous large pustules of stem rust, *Puccinia graminis avenae* Eriks. and Henn., appeared on a considerable number of plants of the resistant oat variety Anthony growing in test plots at the Crookston, Minnesota, Agricultural Experiment Station and in adjacent commercial fields. The same situation developed at Crookston in 1940, although there was very little stem rust on Anthony at the Waseca, Morris, Grand Rapids, and St. Paul stations. Anthony is no longer of commercial importance in Minnesota; but, since this variety has long been moderately to highly resistant to stem rust, it seemed desirable to ascertain whether a new physiologic race of *P. graminis avenae* had appeared or whether the unusual development of rust might have been due to the effects of extraordinary weather or soil conditions or to varietal mixture in the oats.

All rust collections made from susceptible plants were identified by Wm. Q. Loegering[2] as *Puccinia graminis avenae*, race 5, to which Anthony always has been resistant as indicated by the production of small uredia surrounded by pronounced chlorotic or necrotic areas.[3] As the race 5 was consistently isolated from large uredia on susceptible plants, and as it is one of the races of oat stem rust most prevalent in the Mississippi Valley, it was clear that no new race was involved.

It seemed improbable that the susceptibility of Anthony at Crookston could have been due to ordinary varietal mixtures because the seedlots used for that station and the other stations previously mentioned had all been supplied by the central station at St. Paul. Samples of the rusted plants from Crookston were examined by H. K. Wilson, Division of Agronomy, University of Minnesota, who considered them Anthony plants on their gross morphological characters. Since the rust-susceptible plants were morphologically indistinguishable from the rust-resistant ones, it seemed possible that the unusual soil or climatic factors might have predisposed plants to rust.

To test the effect of meteorological conditions, seedlings of Anthony, grown from the 1939 seed lot[4] of University Farm and the same that was sent to Crookston for planting in 1940, were inoculated with race 5 of *Puc-*

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[1] Assistance in the preparation of these materials was furnished by the personnel of the Work Projects Administration, Official Project No. 165-71-1-124, sponsored by the University of Minnesota. Paper No. 1990, the Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Agent, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.

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[4] Seed lot supplied by H. K. Wilson, Division of Agronomy, University of Minnesota.

cinia graminis avenae under varying conditions of light, temperature, atmospheric moisture, and soil moisture; but there were no consistent differences in rust development under the various conditions. Occasional plants, however, became heavily rusted in all the tests. The tests were repeated several times, with the same results: there was no association between particular environmental conditions and the number of abundantly infected plants. The number of susceptible Anthony plants produced by seedlots grown at the several experiment stations of the University of Minnesota, however, varied somewhat. In one experiment, of 20 seedlings from seed grown at Waseca in 1938, 15 plants were susceptible and only 5 resistant. Seed lots from other branch stations, nevertheless, did not produce so many susceptible plants; and of a total of 550 seedlings inoculated, only 16 per cent were susceptible.

Because of the lack of positive information from the greenhouse tests and because susceptible plants of Anthony were found in the field only at Crookston, experiments on the possible effect of fertilizers were made at Crookston and St. Paul in 1941. The trial plots included fertilizer treatments with treble superphosphate at 100 lb. an acre, treble superphosphate at 100 lb. an acre plus potash at the same rate, and 3 minor-element treatments with boron at 15 lb., manganese at 25 lb., and zinc at 25 lb. an acre. Plots were arranged as in diagram 1, treatments being made separately and also as combinations of fertilizers and minor elements.

Diagram 1. *The arrangement of fertilizer plots for Anthony oats at Crookston and at St. Paul, Minnesota, in 1941*

Phosphate Boron	Phosphate	Phosphate Manganese	Phosphate Zinc	Phosphate	Phosphate Boron Manganese Zinc
Boron	No treatment	Manganese	Zinc	No treatment	Boron Manganese Zinc
Phosphate Potash Boron	Phosphate Potash	Phosphate Potash Manganese	Phosphate Potash Zinc	Phosphate Potash	Phosphate Potash Boron Manganese Zinc

So little stem rust, only 5 per cent, developed in Crookston plots in 1941 that no differences were observable. At St. Paul 15 per cent stem rust developed, but there were no differences either in plant development or in stem-rust infection in the different plots. However, about 15 per cent of the plants were severely rusted (50 to 60 per cent) at St. Paul and between 10 and 15 per cent in the plots at Crookston; and, again, race 5 of *Puccinia graminis avenae* was identified from 4 collections from the St. Paul plots and 2 collections from the Crookston plots. The susceptible plants at St. Paul were examined by H. K. Wilson, Division of Agronomy, University of Minnesota, who considered them indistinguishable from Anthony.

Susceptible and resistant seedlings of Anthony that had been inoculated with race 5 in the greenhouse were transplanted to the field and grown to maturity. The seedling reaction (Fig. 1) was maintained in the adult plants, the susceptible seedlings giving rise to plants with numerous large pustules, the resistant seedlings to plants with small uredia and indications of necrosis. There was 50 per cent rust on the susceptible plants.

Seed was saved from susceptible and resistant plants in the above tests and, similarly, from susceptible and resistant plants in the fertilizer tests. Seedlings grown from this seed were inoculated in the greenhouse with race 5 of *Puccinia graminis avenae* collected from susceptible Anthony at University Farm, St. Paul, in 1941. In all cases seed from susceptible plants produced susceptible seedlings and that from resistant plants, resistant seedlings.

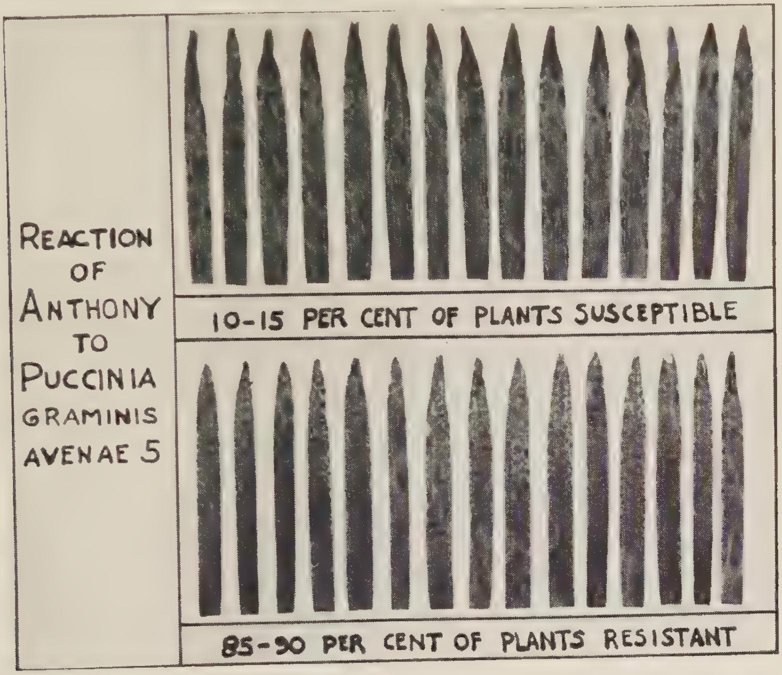
REACTION OF ANTHONY TO PUCCINIA GRAMINIS AVENAE 5
10-15 PER CENT OF PLANTS SUSCEPTIBLE
85-90 PER CENT OF PLANTS RESISTANT

FIG. 1. The reactions of seedlings of Anthony oats (C. I. 2143) to race 5 of *Puccinia graminis avenae* and the proportions of susceptible and resistant plants in field plots in Minnesota.

It appears, therefore, that there are at least 2 strains of Anthony oats, indistinguishable morphologically but differing in reaction to stem rust race 5, (Fig. 1). The percentage of susceptible plants in the seedlots tested is about 10 or 15 per cent. About 15 per cent of a total of 500 adult plants were susceptible in field plots at St. Paul. There is no doubt that the seed lots of Anthony oats in use in Minnesota contain many plants susceptible to at least one of the prevalent races of oat stem rust.

Spectrographic analyses[5] were run on the resistant and susceptible

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[5] We are indebted to Richard C. Nelson, Philip Hamm, and David Gottlieb for the spectrographic analyses.

Anthony seedlings to see if they differed in ability to absorb from the soil certain elements that might affect reaction to rust. Densitometer readings for the magnesium and iron lines for the 2 strains of Anthony were nearly identical, and inspection of the lines for other emission elements on the film revealed no differences between the resistant and susceptible plants.

In conclusion it seems advisable in the case of varieties that have been bred for resistance to disease to practice periodic reselection within the variety. Resistant varieties should be exposed to artificial epidemics of diseases to make possible the observation of any variation in disease reaction and to eliminate segregates or, even, mutants that are not pure for the resistant character. Strains are known to exist within other cereal varieties and to differ with respect to disease reaction. Renown wheat was not pure for resistance to leaf rust, *Puccinia rubigo-vera tritici* (Eriks. and Henn.) Carl., but reselection within this variety produced a highly resistant strain.[6] The hard red winter wheat, Crimean C. I. 1435, is considered by Kiesselbach and Peltier[7] a mass variety; they demonstrated that the majority of 578 of its most promising strains fell into 3 different groups on the basis of their reaction to selected races of *P. graminis tritici* Eriks. and Henn. In contrast to our experience with Anthony oats, many of the strains of Crimean differed from each other agronomically, as well as in rust reaction. However, in Kanred, a stem-rust resistant selection from Crimean, Kiesselbach and Peltier found a few individuals susceptible to a race of stem rust that, ordinarily, did not attack Kanred. Johnston[8] also noted the existence within supposedly pure wheat varieties of strains indistinguishable morphologically but differing in reaction to disease. He worked with approximately 200 soft red winter wheat varieties that were ordinarily susceptible to race 9 of leaf rust, *P. rubigo-vera tritici;* and in 28 of them he found strains resistant to leaf rust. Thus he believed that selection within a variety was an excellent means of obtaining rust resistant strains. And from another standpoint, it is probable that many disease-resistant crop plants need periodic reexamination, exposure to artificial epidemics of disease, and reselection within the variety to ensure the maintenance of its resistance.

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PHYTOPATHOLOGICAL NOTES

New Fungicides and Reduced Fungicide Dosages for the Control of Kernel Smut of Sorghum.—During the 1942 season, four new fungicidal seed treatment materials and several previously used were tested for their effect on emergence in sorghum, and on the control of covered kernel smut at full, half, and fourth dosages.

The following materials were used: New Improved Ceresan, 5 per cent ethyl mercuric phosphate (Bayer Semesan Co.); copper carbonate, 54 per cent metallic copper; Spergon, 99 per cent tetra-chloro parabenzoquinone (U. S. Rubber Company); Thiosan (DuBay 1205–FF), 50 per cent tetramethyl thiuramdisulphide; and DuBay 870, 100 per cent ferric dimethyl dithio carbamate (Bayer-Semesan Company); Captax, 100 per cent mercaptobenzo-thiazole (R. T. Vanderbilt Company); Sanoseed, 2.2 per cent ethanol mercuric chloride (Ansbacher Siegle Corporation); M.T.D.S., morpholine thiuram disulphide (M. C. Goldsworthy); and dusting sulphur (Stauffer Chemical Company).

Seed of Sharon kafir was dusted with spores of covered kernel smut at the rate of 1 g. of spores to 100 g. of seed; separate portions were then treated with New Improved Ceresan at the rates of $\frac{1}{2}$, $\frac{1}{4}$, and $\frac{1}{8}$ oz. per bu., and with the other dusts at 3, $1\frac{1}{2}$, and $\frac{3}{4}$ oz. per bu.

The treated seed, along with similarly smutted untreated seed, was planted at Beltsville, Maryland, at the rate of 200 seeds per 44-foot row, replicated 4 times for each rate of application of each fungicide. Similar plantings were made at 7 other stations in the Midwest and Southwest, 3 replications per location.

Data on emergence were obtained at 5 stations and on smut control at 7. At Hays and Manhattan, heavy rains caused irregular emergence; at Tucson, high temperature inhibited smut development. The results are summarized in table 1.

On the whole, Thiosan, DuBay, 870, copper carbonate, and Spergon seemed most consistently beneficial to emergence, while Sanoseed and sulphur were of little, if any, benefit in this respect. Spergon controlled smut perfectly Thiosan and DuBay 870 reduced it to an average of less than 0.1 per cent at all dosages, while M.T.D.S. and copper carbonate were only slightly less effective. Captax and sulphur were fairly effective at full dosage, and were superior to New Improved Ceresan, which was 2 years old and evidently had deteriorated. Sanoseed was highly ineffective in smut control.

Smut in the untreated checks ranged from 7.8 to 43.4 per cent and averaged 25.2 per cent and hence the fungicides were not put to a uniformly severe test. However, the excellent smut control obtained with the new materials, Thiosan, DuBay 870, and M.T.D.S. at all dosages, and with Captax at the maximum dosage, indicates their possible effectiveness in controlling certain diseases of other field crops. If so, they can be substituted for those

TABLE 1.—*Effect of seed treatment with different fungicides at three rates of application, on seedling emergence of and on occurrence of covered kernel smut on Sharon kafir at several stations, 1942*

Treatment		Percentage emergence[a] and percentage heads smutted at												Totals and averages			
Fungicide	Oz. per bu.	Beltsville, Md.		Lincoln, Nebr.		Garden City, Kans.		Stilwater, Okla.		Dalhart, Tex.		Man-hattan, Kans.	Hays, Kans.	Av. Emer.	Heads		
		Emer.	Smut	Emer.	Smut	Emer.	Smut	Emer.	Smut	Emer.	Smut	Smut	Smut		Total No.	Smutted	
																No.	Pct.
New Improved Ceresan[b]	½	54	1.8	70	3.3	73	1.6	69	0.3	61	2.4	1.0	0.0	65	2088	34	1.63
	¼	57	11.2	69	8.1	70	4.9	82	1.8	57	3.5	2.5	1.6	67	2266	120	5.30
	⅛	57	9.8	50	20.6	65	11.2	92	3.2	55	12.4	11.2	1.1	64	2164	195	9.01
Copper carbonate	3	59	0.0	78	0.0	71	0.7	69	0.0	67	0.0	0.0	0.0	69	2300	3	0.14
	1½	61	0.0	74	0.0	73	0.5	86	0.0	59	1.3	2.0	0.0	71	2173	8	0.37
	¾	58	0.7	58	0.7	74	0.5	80	0.0	63	0.9	0.0	0.0	67	2126	10	0.47
Spergon	3	62	0.0	54	0.0	78	0.0	85	0.0	60	0.0	0.0	0.0	68	2410	0	0.0
	1½	60	0.0	63	0.0	73	0.0	85	0.0	69	0.0	0.0	0.0	70	2416	0	0.0
	¾	57	0.0	49	0.0	70	0.0	94	0.0	56	0.0	0.0	0.0	65	2410	0	0.0
Thiosan	3	64	0.0	75	0.0	69	0.0	80	0.0	68	0.0	0.0	0.0	71	2486	0	0.0
	1½	63	0.0	74	0.0	68	0.0	93	0.0	60	0.0	0.0	0.0	72	2162	0	0.0
	¼	53	0.2	64	0.0	63	0.0	88	0.0	59	0.0	0.0	0.0	65	2221	1	0.05
DuBay 870	3	68	0.4	77	0.0	65	0.0	83	0.0	66	0.0	0.0	0.0	72	2505	2	0.08
	1½	59	0.4	72	0.0	70	0.0	90	0.0	65	0.0	0.0	0.0	71	2407	2	0.08
	¼	55	0.2	53	0.0	61	0.0	91	0.0	65	0.3	0.0	0.0	65	2189	2	0.09
Captax	3	58	0.0	66	0.0	71	0.5	81	0.0	61	0.0	0.0	0.0	67	2218	2	0.09
	1½	55	2.9	59	1.2	63	0.8	79	0.0	62	0.6	0.7	0.0	64	1985	21	1.06
	¾	51	9.1	53	5.1	58	1.9	70	1.4	58	3.5	5.3	0.5	58	1815	73	4.02
Sanoseed	3	57	13.2	58	13.4	52	6.1	80	4.8	54	13.2	11.9	6.9	60	2119	207	9.77
	1½	46	25.6	54	26.3	52	18.9	81	9.2	50	17.8	38.4	0.0	57	2010	365	18.16
	¾	45	17.3	43	43.2	54	12.7	86	9.8	47	28.6	35.3	5.6	55	1956	360	18.40
M.T.D.S.	3	57	0.9	59	0.0	57	0.3	77	0.0	63	0.0	0.0	0.8	63	2101	7	0.33
	1½	59	0.2	62	0.0	62	0.0	85	0.0	57	0.0	0.0	0.0	65	2077	1	0.05
	¾	54	2.2	63	0.0	52	0.8	86	0.0	53	0.3	0.0	1.0	62	2011	15	0.75
Sulphur	3	44	2.3	71	0.0	51	0.3	77	0.0	44	0.3	0.0	0.5	57	1995	10	0.50
	1½	51	1.3	59	3.9	50	1.5	85	0.0	45	0.0	0.0	0.0	58	1882	19	1.01
	¾	50	1.2	61	0.0	47	2.6	82	0.0	52	0.3	1.9	0.0	58	2156	28	1.30
None	.. ..	49	34.4	64	33.5	57	12.9	78	13.0	51	25.2	31.1	7.8	60	1969	442	22.45
	......	48	40.7	56	31.5	57	25.8	73	15.0	40	29.5	15.9	10.5	55	1718	471	27.42
	. . .	52	33.3	61	43.4	51	20.3	77	17.3	45	25.9	25.4	11.7	57	2013	519	25.78

[a] Emergence data were not obtained at two stations because of washing.
[b] Made in 1940 and apparently deteriorated.

fungicides containing metals highly essential in the manufacture of armaments. Thiosan is on the market as a fungicide for turf, while Captax is used in the manufacture of rubber. DuBay 870 and M.T.D.S. are still in the experimental stage. R. W. LEUKEL,[1] Bureau of Plant Industry Station, Beltsville, Md.

Epichloe typhina on Imported Fescue Seed.—In a search for stoloniferous fescues suitable for airfields, fairways, and playing surfaces, the department of agronomy at this station imported seed of two strains of *Festuca rubra genuina* and one of *F. rubra nemoralis* from Hungary in 1939, as follows:

Strain 1. *F. rubra genuina* via T. W. Woods & Sons, Richmond, Virginia
‘‘ 2. *F. rubra nemoralis* ‘‘ ‘‘ ‘‘ ‘‘ ‘‘ ‘‘ ‘‘ ‘‘
‘‘ 3. *F. rubra genuina* ‘‘ Benö Bálint & Sons, Budapest, Hungary

Seed was planted in flats in the greenhouse and approximately 250 individual plants of each strain were transferred to the nursery for observation and subsequent selection. Strain 3 showed the greatest variation in growth habits and types but individual plants from all strains were chosen for further study. These plants were divided to give 20 plant clones and transferred to another location for further observation on growth habit, seed yield, foliage color, disease resistance, etc. In June of the current year these clones were in their second season of seed production.

Of 104 selections of strain 3, 27 clones showed this year fruiting structures of *Epichloë typhina*. In a few clones every seed stalk had the typical sign of the ‘‘choke’’ disease, bearing white stromata on the leaf sheaths and occasionally on some of the spikelets of the panicle. Often the panicle failed to emerge. In other clones at least one plant showed evidence of infection.

According to Sampson and Westen[1] this fungus is systemic in *Festuca rubra* and is transmitted through the seed in percentages as high as 99; infected plants clonally divided eventually show disease in every plant of the clone. Plants systemically infected may produce a hundred or more panicles, yet show nothing of the parasite to the casual observer.

In lieu of this information all infected clones were removed from the nursery and burned. A few badly infected plants were removed to an isolated plot and interplanted with noninfected plants in an effort to establish rate of spread under field conditions. Seed from these infected plants was collected for the purpose of studying seed transmission and the effect of hot-water treatment on seed disinfection. It is doubtful if these studies will yield pertinent information until three years hence.—C. C. WERNHAM, Dept. of Botany, The Pennsylvania State College, State College, Pa.

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[1] The generous cooperation of the following investigators at these stations is gratefully acknowledged: J. E. Livingston and R. L. Cushing at Lincoln, Nebr., A. E. Lowe at Garden City, Kans., E. G. Heyne, at Manhattan, Kans., A. F. Swanson at Hays, Kans., J. B. Sieglinger and D. E. Hoffmaster at Stillwater, Okla., A. T. Bartel at Tucson, Ariz., and B. F. Barnes at Dalhart, Tex.

[1] Sampson, K., and Western, J. H. Diseases of British grasses and herbage legumes, 26–29. 1941. Cambridge Univ. Press.

An Apple Rot Fungus Morphologically Related to a Human Pathogen.—A rot of stored apples, attributed to *Sporotrichum malorum* by Kidd and Beaumont,[1] was brought to the attention of the writer in 1937, when this disease was observed causing serious losses in stored Winesap apples in Virginia. Kidd and Beaumont,[1] in England, reported it as new in 1924, and named the causal fungus *Sporotrichum malorum.* Gardner[2] reported that what he considered a strain of the same fungus caused a decay of Grimes Golden apples in Indiana in 1929. In 1931 Ruehle[3] in a report of "New Apple Rot Fungi from Washington" listed *Sporotrichum malorum* Kidd and Beaumont and also described a new species, which he designated as *S. carpogenum.*

Since 1937 the writer has isolated similar fungi from decaying apples, from cankerous apple wood, and from the surface soil beneath apple trees. Instead of finding the isolates to be either identical with or distinctly different from the published species, the writer observed a wide range of variation in the 130 single-spore isolates studied. Preliminary comparison of the isolates with a sub-culture of *S. malorum* obtained from the Centraal Bureau vor Schimmelcultures indicated that the original classification was in error. The present study, therefore, has been made in an effort to determine the proper systematic position of the fungus described by Kidd and Beaumont as *Sporotrichum malorum,* and that described from a similar apple rot by Ruehle as *S. carpogenum,* and to find the relationship between these fungi and the many variable isolates obtained during the course of this study.

It has been demonstrated that this fungus has dark pigment in the hyphae and to a certain degree in the spores; therefore, it should be placed in the Dematiaceae instead of in the Moniliaceae. Since the process of sporulation is characterized by a continuous proliferation of the sporogenous cells, forming conidia through a collar-like apex, where they abscise but are held together in a mucous mass, it seems evident that this fungus does not belong in the form-genus *Sporotrichum.* It, therefore, is being transferred to the form-genus *Phialophora,* and *Sporotrichum malorum* Kidd and Beaumont becomes *Phialophora malorum* (Kidd and Beaumont) comb. n. *Sporotrichum carpogenum* becomes synonymous with *P. malorum* and is considered to be a strain of that species.

The form-genus *Phialophora,* as established by Thaxter (in Medlar),[4] is characterized by spore formation in cups borne on the tips of flask-shape conidiophores. *P. verrucosa* was designated as the type species and at that time was the only species of the new form-genus. This fungus had been isolated from a skin disease, reported as the first case of *dermatitis verrucosa*

[1] Kidd, M. N., and A. Beaumont. Apple rot fungi in storage. Trans. Brit. Mycol. Soc. 10: 98–118. 1924.

[2] Gardner, Max W. Sporotrichum fruit spot and surface rot of apple. Phytopath. 19: 443–452. 1929.

[3] Ruehle, George D. New apple rot fungi from Washington. Phytopath. 21: 1141–1152. 1931.

[4] Medlar, E. M. A new fungus, *Phialophora verrucosa,* pathogenic for man. Mycologia 7: 200–203. 1915.

(*chromoblastomycosis*), a skin disease of man. The apple-rotting fungus aligns itself with the form-genus *Phialophora*, strictly in a morphological sense, because of the unique conidial formation. There has been no evidence of any relationship between the diseases that these fungi cause. Apples inoculated with *P. verrucosa* remained sound, and, even under semi-saprophytic conditions, the fungus did not grow or cause any disintegration of the apple tissue. In 1937 Conant[5] concluded that the form-genus *Cadophora*, with its specific forms reported as causing bluing and staining of wood pulp, is synonymous with the form-genus *Phialophora*. Inasmuch as *Phialophora* had priority, he proposed new combinations for the described species. The transfer of the apple-decaying species with its numerous strains to *Phialophora malorum* is a step further in showing the wide distribution of this form-genus in nature.—L. P. MCCOLLOCH, Bureau of Plant Industry Station, Beltsville, Md.

Willow Blight in West Virginia.[1]—In 1941 Leach and Rupert[2] reported willow blight in West Virginia near Thomas in Tucker County. It was observed only on trees of an ornamental planting. The owner stated that the trees had been obtained from an out-of-State nursery, but the exact origin could not be determined, as the records of purchase had been lost. The disease has since been found in limited areas on native willows in two adjacent counties, but it is not widely distributed. All infections on native willows apparently are of relatively recent origin, as there are few dead twigs or old cankers. It seems probable that the disease has spread to native willows from the planted stock.

The frequent association of *Physalospora miyabeana* Fuk. and *Fusicladium saliciperdum* Tub. in willow blight has led to some dispute as to which is the primary parasite. The disease was first reported in this country by Clinton[3] and Clinton and McCormick,[4] who regarded *F. saliciperdum* as the causal agent. In England Nattrass[5] and Dennis[6] regarded *P. miyabeana* as the primary parasite and relegated the *Fusicladium* to the rôle of a secondary invader. Harrison[7] in Canada and Brooks and Walker[8] in England concluded that both organisms are responsible for injury.

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[5] Conant, Norman F. The occurrence of a human pathogenic fungus as a saprophyte in nature. Mycologia 29: 597–598. 1937.

[1] Published with the approval of the director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 293.

[2] Leach, J. G., and J. A. Rupert. Black canker of willow in West Virginia. U. S. Dept. Agr. Pl. Dis. Rptr. 25: 588. 1941.

[3] Clinton, G. P. A new disease of willows appears in Connecticut. U. S. Dept. Agr. Pl. Dis. Rptr. 11: 87–88. 1927.

[4] Clinton, G. P., and F. A. McCormick. The willow scab fungus. Conn. Agr. Exp. Stat. Bull. 302. 1929.

[5] Nattrass, R. M. The Physalospora disease of the basket willow. Trans. Brit. Mycol. Soc. 13: 286–304. 1928.

[6] Dennis, R. W. G. The black canker of willows. Trans. Brit. Mycol. Soc. 16: 76–84. 1931.

[7] Harrison, K. A. Willow blight. Canada Dept. Agr. Rept. Dom. Bot. (1928): 34–36. 1929.

[8] Brooks, F. T., and M. M. Walker. Observations on *Fusicladium saliciperdum*. New Phytol. 34: 64–67. 1935.

When the disease was first observed in West Virginia the season was well advanced and only *Physalospora miyabeana* was observed associated with blighted twigs, but in May, 1942, *Fusicladium saliciperdum* was found fruiting abundantly on infected petioles. No acervuli of the *Physalospora* were present then, although the fungus could be isolated from the cankers. In June, acervuli of *P. miyabeana* were found to be very active, while the *Fusicladium* was definitely on the decrease. Perithecia of *P. miyabeana* were found in some of the cankers in late summer and in the winter.

Pure-culture inoculations showed the *Physalospora* to be pathogenic on both stems and leaves of growing trees, in the greenhouse and out of doors, as well as on excised leaves in a Petri dish. The *Fusicladium*, however, under comparable conditions, failed to infect the stems and leaves of growing trees. On excised leaves it produced only a weak, delayed infection after the leaves had begun to deteriorate.

In nature there was always a sudden appearance of a saprophytic species of *Cladosporium* on the affected leaves very soon after they began to die. It is probable that the *Fusicladium* may act somewhat similarly, although there are some indications that it may be weakly parasitic. However, in these experiments it was not nearly so virulent as *Physalospora*.

A species of *Macrophoma* was occasionally found fruiting in old cankers and dead twigs but this fungus was never isolated from blighted leaves or twigs in the early stages of infection. It, also, is most likely a secondary invader.—JOSEPH A. RUPERT and J. G. LEACH, Department of Plant Pathology and Bacteriology, West Virginia University, Morgantown, W. Va.

Longevity of the Spores of Some Wood-destroying Hymenomycetes.—Fresh sporophores of each fungus were placed in a moist chamber for 12 to 24 hours to deposit their spores on clean glass slides. These spore prints were allowed to dry at room temperature before wrapping in tissue paper for storage at room temperature. At approximately weekly intervals spores were sown on slants of Sabouraud's dextrose agar. A No. 30 platinum wire with a loop about 1 mm. in diameter was flame-sterilized. A drop of sterile water picked up in this loop was used to rinse the spores from a small area on the slide. The spores were streaked out on the agar slant, 3 such loopfuls being transferred to each tube. The moistened area on the spore print was marked on the reverse of the slide with wax pencil, and thereafter avoided in securing spores for further sowings. After the spot on the slide was dry, the latter was returned to its tissue-paper wrapping.

The tube was capped with a double thickness of wax paper and held at room temperature. Growth was observed with the help of a 10 × hand lens. With proper care to obtain spores from healthy sporophores, bacterial or mold contaminations were rare.

Stereum hirsutum spores were alive after 56 but not after 64 days of storage. Growth was distinctly visible at the end of the first week.

Stereum rugisporum spores were alive after 46 but not after 66 days.

At least two weeks were required for visible growth. The sowings made after 16 and 38 days failed to show growth.

Stereum sanguinolentum spores were alive after 131 days but not after 137 days. Growth was visible after 4 or 5 days. Only one earlier sowing, that after 101 days, failed to grow.

Polyporus schweinitzii spores were alive after 162 but not after 170 days. Growth was visible after 2 or 3 weeks.

Fomes igniarius spores were alive after 91 but not after 99 days. Growth was visible after 20 to 27 days. Germination was somewhat erratic, fresh spores and those stored 7, 14, and 64 days failing to germinate.

Fomes pinicola spores were alive for at least 173 days. Growth was visible after 5 to 7 days.

Trametes pini spores were alive for at least 65 days. About 2 weeks were required for growth to become visible.

Polyporus abietinus spores were alive for at least 65 days. About 1 week was required for growth to appear.

Pleurotus ostreatus spores were alive at least 20 days. Growth was visible in about 1 week.

In an earlier experiment, *Coniophora sistotremoides* spores grew when sown on casein-glucose-potato agar after 46 but not after 68 days. At first growth appeared in 17 or 18 days, but the last two successful sowings required about 50 days to make visible growth.

Hymenochaete tabacina spores grew on walnut nutrient agar after being dry for 17 days. Growth was visible in 4 or 5 days.

These rather fragmentary results are reported at this time, since the work is necessarily suspended for the duration of the war.—CHARLES H. HARRISON, 2318 N. 38 Street, Seattle, Washington.

BOOK REVIEWS

Karling, John S. (Columbia University). *Plasmodiophorales.* 144 p., 17 pl., 17 figs. Publ. by the author (New York) 1942.

Had this book emanated from a government bureau it would doubtless have been seized upon by a Congressional committee investigating useless expenditures as an example of the sort of activity that must be suppressed in time of war, notwithstanding that it contains some references to diseases of economic crops. On the other hand, it is likely to be hailed by mycologists who have not completed their transfer of allegiance to Mycology's domineering offspring, Phytopathology, as another volume in the series of American monographs that illuminates minutely a corner of the realm of fungi for the sake of knowledge itself—a series distinguished by such names as Thaxter, Burt, Fitzpatrick, Coker, and Couch. The present work exhibits less of the author's personal handiwork than some of its predecessors but is still an authoritative treatment of a field in which specialists are rare.

Its scope is roughly indicated by the table of contents as (1) Introduction, (2) Cytology, (3) Sexuality and alternation of generations, (4) Classification and description of species, (5) Phylogeny and relationships, and (6) Diseases caused by species of the group. In the discussion of ''promitosis'' and the ''akaryote stage'' the author stresses the finding by recent authors, employing improved technique, of chromosomes in the vegetative divisions, and their failure to find convincing evidence of an akaryote condition. Thus these features, which have been held to relate the Plasmodiophorales to the Myxomycetes and Protozoa, are gravely suspected of not occurring in the group at all.

The author has consistently maintained a judicial attitude toward the evidence, often fragmentary, on the cytology and life cycle of the organisms that different investigators have assigned to this group, and the evidence in figures and in text is carefully reviewed, even when the claim to relationship is rejected. He recognizes 8 valid genera, as compared with 5 by Fitzpatrick in *The Lower Fungi*, 1930, but 2 of these have been described since the latter book appeared. He regards the group as belonging to the primitive fungi with its closest affinity to the Woroninaceae, though of questionable relationship to the other families usually included in the Chytridiales. In postulating this relationship the presence of biflagellate heterocont zoospores in the Plasmodiophorales is emphasized. He does not view the evidence regarding sexuality and alternation of generations in the group as warranting general acceptance. The reviewer feels that the chapter entitled Introduction is more helpfully read at the end since it provided a needed synthesis after he had lost his bearings in navigating the many details of the 4 ensuing chapters. He also found it helpful, in gaining a quick conspectus of the group, to add symbols to the index of species on p. 137 differentiating the valid genera and species from the others; a slight modification of typography would have gained the same end.

The final chapter gives very full information on club root of crucifers and powdery scab of potatoes, including (for the former) a complete host list, a table of effects of various fungicides and crop ameliorants on the pathogen, and lists of varietal reactions. Separate bibliographies pertaining to the subject matter of each chapter are provided. The illustrations are numerous and well executed.—Freeman Weiss.

Chester, K. Starr. *The Nature and Prevention of Plant Diseases.* I–XII, 1–584. The Blakiston Company. 1942. $4.50.

The publication of a new textbook in plant pathology immediately raises the question as to the reason for its publication. Is it the result of a definite need for something better; and if so, does the new text supply the need? In the preface of this new book, the author states that one of his objectives is to provide the student with a work to which he may refer for detailed and specific directions on plant-disease control; and follows with a statement that ''I have tried to select for detailed study diseases that are of considerable economic importance over a broad area of the United States . . . and that illustrate the leading principles of plant pathology.'' Also an attempt is made to ''rectify the past neglect of diseases of southern and prairie crops.''

The author seems to have met these objectives fairly successfully. The selection of subjects is good and should meet general approval, particularly the discussion of a larger number of diseases caused by the imperfect fungi. The arrangement of the text is somewhat different from that of some other texts, but not radically so. It begins with a chapter on ''The significance of plant disease in agriculture.'' This is rather a thrilling statement of the losses farmers sustain year after year as a result of outbreaks of diseases in their crops. At the same time, the author works in a very good limited history of plant pathology. In the second chapter, types of plant diseases are outlined, with the remainder of the chapter devoted to a discussion of the fungi, their classification and types of fruiting bodies, and infection by fungi. The next 7 chapters are devoted to diseases caused by the fungi, the arrangement being as follows: Basidiomycetes, Rusts; Smuts; Fleshy fungi and Mycorrhizae; Ascomycetes; Imperfect fungi; Phycomycetes; and Damping-off and related troubles. These are followed by chapters on Bacterial diseases; Virus diseases; Parasitic seed plants and algae; Nematodes; Physiogenic diseases; Methods of studying plant diseases; Environment and parasitic disease; Etiology and epiphytology of disease; and three chapters on principles and procedures in the control of plant diseases: 1, by regulation, 2, by inducing resistance, and 3, by cultural methods. This is followed by a rather complete index.

The author has discussed the majority of the most destructive or, at least, the better-known plant diseases and has also included in each group a number of minor diseases; for example, 21 rusts are treated, including rusts of grasses, forage crops, trees and shrubs, vegetables, and ornamentals. The diseases are given space according to the economic losses they cause. Seventy-two pages are devoted to the diseases caused by fungi imperfecti, with a discussion of 24 well-selected diseases. A feature of the chapter on damping-off and related troubles is the discussions of disease complexes, as the cotton-seedling blight and boll-rot complex; corn root, stalk, and ear rots; and sorghum root and stalk rots. The author is at his best in the field with which he is most familiar, namely, the diseases caused by viruses. If the text is widely adopted, a great impetus will have been given to the use of binomials in naming viruses, because it is accepted without reservation. In the chapter on physiogenic disease, less than a page is devoted to the general symptoms produced in plants by a shortage of N, P, and K. Without headings it would be difficult for one experienced in nutrient deficiencies to guess which deficiencies were being described. Photographs of typical nutrient deficiencies would add greatly to the value of this part of the discussion.

A successful textbook in plant pathology will be the chief source of information on plant diseases for numerous students, both in class and in the field later on, and for an occasional one it will form the ground work upon which he will build his knowledge of plant diseases as a profession. Each group has a right to expect, in a text, a high degree of scholarship with as nearly absolute accuracy as the literature will provide; and where all of the facts are not known, a high degree of judgment in the interpretation of the available facts. While the text is a well-arranged, well-balanced discussion of plant diseases for the beginner, yet, an occasional mistake makes one lose a certain degree of confidence not only in the discussion in which it occurs but in the discussion of those topics with which one is not too familiar. For example, the author has the ''double-sexed mycelium'' of stem rust, following fertilization of the receptive hypha in the pycnium, ''growing vigorously down into the barberry leaf,'' and feeding ''on its cells'' (p. 38); and teliospores ''fall to the ground,'' resist winter temperatures, and the following spring germinate, etc. (p. 39). It is doubtful whether the scab fungus invades the corn stem and causes barrenness; or that in the southern States the corn root-rot stage (caused by the scab fungus) is common (p. 108); or that the powdery mildew fungi produce toxins that pass through the leaf tissue and yellow and kill cells (p. 135). The powdery mildew epidemic of red clover in 1922 is commented upon as though the fungus were of long standing in this country, but flared up suddenly that year and then subsided, rather than pointing out that it was a new introduction at about that time from Europe and has been injurious each year since its introduction (pp. 136, 452). What is meant by the statement that the sexual overwintering stage of *Sclerotinia fructicola* was determined in 1909? Surely not first discovered and described that year (p. 138). In speaking of the peach leaf-curl fungus the statement is made that ''it oversummers and overwinters as ascospores on the bud scales,'' and ''in the leaf curl fungi saprogenesis is wanting in nature but may be experimentally produced on culture media.'' Actually, there seems to be no evidence that the ascospores play any necessary part whatever in the life history of the fungus, once it is established in a peach orchard; but the yeast-like saprophytic stage appears to perpetuate the fungus year after year (pp. 147, 466). In control of the Texas root-rot fungus, it is claimed that, on small areas, soil disinfectants, such as 1.25 per cent formaldehyde or 0.25 per cent organic mercury, may be used to eradicate the fungus, but the disinfectant must penetrate to a depth of 4 feet. Actually, formaldehyde is completely filtered out of solution in passing through about 4 inches of soil (p. 179). The spread of the peach scab-fungus during the growing season usually is attributed to water rather than ''by means of wind-blown conidia'' (p. 196).

The false but widespread belief that southern anthracnose has been the principal limiting factor in red clover production in Tennessee and Kentucky and surrounding States is repeated by the author, although it is not supported by the literature. According to the usually accepted definition, *Phyllosticta solitaria* would be considered a facultative saprophyte and not a facultative parasite (p. 212). The same mistake is made elsewhere in the text. Sweet-potato ring rot is not caused by *Rhizopus nigricans* but by *Pythium ultimum* (p. 237). It is questionable whether bruised fruits and vegetables, held in an environment favorable to the fungus, *invariably* develop *Rhizopus* infection. The toxin secreted by the wildfire organism does not seem to be responsible for the necrotic reaction in tobacco, as is claimed (p. 284). There seems to be a fair consensus of opinion that zinc-lime spray does not control bacterial spot of peach, but does reduce arsenic injury (p. 296). While it may be true that "alfalfa wilt is the most important disease of the crop," the statement would be definitely misleading to students in certain alfalfa-growing regions of the U. S. in which the disease rarely occurs (p. 310). The watermelon usually is considered nearly immune from the cucurbit bacterial-wilt pathogen and not susceptible, as the author's statement would imply (p. 312). It is rather the exception than the rule that "chlorophyll in leaves formed subsequent to inoculation" with a mosaic virus "is lacking in certain leaf areas." Chlorophyll may be suppressed to a certain extent but patterns are usually shades of green. It is not true that the tobacco mosaic "easily stands the heating involved in the curing process." In flue-curing, temperatures are commonly reached that destroy the virus completely in certain parts of the curing shed (p. 329). In the discussion of acquired immunity, ring-spot of tobacco is given as the classical example of a plant that recovers and develops immunity and shows no further symptoms. Pollen sterility and symptoms produced in "recovered" plants when the temperature is reduced are disregarded. "Immunity is absolute, a plant is immune or not immune from a pathogen," and immunity is defined as "freedom from disease because the qualities for development of the pathogen are lacking in the plant," and yet plants with acquired immunity all support the parasitic virus (pp. 330, 490). A millimicron does not, as the author claims, $= 1\ \mu\mu$, which is a micromicron or 1×10^{-12} meter. A millimicron $= 1\ m\mu$ or 1×10^{-9} meter (p. 325). The statement that in ordinary tobacco no symptoms are seen on the inoculated leaf (tobacco-mosaic virus) will hardly stand scrutiny. While it may be true that several species of aphids spread ordinary tobacco mosaic, the evidence for this occurring in the field is extremely meager (p. 344).

The statement that *manganese* sulphate is recommended for control of *magnesium* starvation (p. 393) is evidently a typographical error, of which there are very few in the text (but on p. 481 *in* for *is;* p. 403 *preferable* for *preferably;* p. 203 *Dayton* for *Drayton;* p. 502 *of* for *or;* and p. 456 *pratctically*). It is doubtful whether it is correct to say that phosphorus shortens the vegetative period of plants. This certainly occurs when phosphorus is added to phosphorus-deficient plants, but it merely brings about normal growth. It is more nearly correct to say that phosphorus starvation lengthens the vegetative period (p. 456). What will the student of cold injury say to the statement that "tissues are frozen, the cells explode, and the plant is killed" (p. 409)? The statement that potatoes regularly produce true seed in the north should be qualified to agree with the facts (p. 413).

While the text is well thought out, and for the most part a well-written, well-illustrated book, the erroneous statements greatly detract from its value and raise the question as to whether it meets the high standards of scholarship that American plant pathologists have set up for themselves.—W. D. VALLEAU, Kentucky Agricultural Experiment Station, Lexington, Kentucky.